THE NATURE AND BIOLOGICAL PROPERTIES OF NEWCASTLE DISEASE VIRUS

by

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INTRODUCTION

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Newcastle disease of fowls is world-wide in distribution and constitutes the most serious of all the economic hazards affecting the poultry industry. Since its recognition as a virus infection, intensive research has been pursued in an effort to evolve some practical method of control. Nevertheless the annual loss to the world food supply from this disease still remains inestimably great.

One of the most striking features of this disease from the epizootiological viewpoint has been the locus of initial outbreaks in recently infected countries. In most cases the locus of primary outbreaks has been on the sea coast. This factor has been attributed to the indiscriminate disposal of infected poultry offal from passing ships. However, during the recent epizootic of Newcastle disease in Scotland (1949/50), observations made by the author while engaged in field control suggested strongly that a sea-bird, the cormorant, was the main source of infection. This epizootiological evidence together with animal inoculation experiments substantiating this contention are presented in this thesis.

Another interesting feature of Newcastle disease has been the wide variation in the clinical

picture exhibited from continent to continent. The classical outbreaks originally described in Europe, Asia and Australia are of an acute virus infection of short duration culminating in a 100% mortality. On the other hand the typical American outbreaks occurring under similar conditions appear to be much milder in their clinical manifestation with a mortality figure ranging from 5 to 50%. While the available evidence suggests that the viruses isolated in the different continents are identical immunologically, it would appear that the wide variation in the clinical picture corresponding as it does to a definite geographical pattern is indicative of some variation in the character of the different virus strains.

In the present investigation 8 strains of Newcastle disease virus isolated in different parts of the world are compared. Antigenic analysis of these strains has been attempted by using the techniques of haemagglutination-inhibition, serum neutralisation, and by serum absorption tests, but only slight differences in antigenic composition can be demonstrated. Biological comparison of these virus strains has proved much more fruitful. Marked differences have been noted in their behaviour in embryonated eggs in regard to multiplication of the virus, and development of the haemagglutinating and haemolytic activities. Virulence tests on

hens have also been carried out and there appears to be a direct correlation between the activity of the virus strain in embryo and its pathogenicity in the adult fowl.

A comparative study of the electron microscopy of the strains of Newcastle disease virus was also carried out without showing any structural differences.

As an essential preliminary a review of the literature relating to Newcastle disease is presented.

REVIEW OF LITERATURE

Review of Literature

Original Description

Newcastle disease was first recognised by Doyle (1927) in Great Britain, the first diseased flock being studied at Newcastle-upon-Tyne and for this reason it received the name by which it is best known.

Kraneveld (1926) described a serious disease of poultry in the Dutch East Indies but failed to demonstrate the causative agent. This disease was later shown to be identical with Newcastle disease.

Geographic distribution

Following the original description, reports of a similar disease emanated from different parts of the world.

Picard (1928) reported that in 1926 a new disease of fowls was observed in the vicinity of Batavia in the Dutch East Indies. It spread rapidly and within a year the entire Dutch East Indian Archipelago was affected. The mortality was practically 100% and vast numbers of fowls died.

Rodier (1928) in the Phillipine Islands described what he believed to be a new disease of fowls. It first appeared in Manila and then spread rapidly over a wide area causing the death of at least 50,000 fowls within a few months. His description of the disease suggested strongly that it was similar to the infection present in the

Dutch East Indian Archipelago and England.

Edwards (1928) and Cooper (1931) made references to a highly virulent disease of fowls reported from widely separated areas in India and described it as Ranikhet disease. At the World Poultry Congress (1930) the official Indian delegate stated that this disease had killed off millions of birds in India.

Konni, Ochi and Haschimoto (1929) investigated an infection of fowls known as Korean poultry disease and they concluded it was Newcastle disease. The disease was caused by a filterable virus and was invariably fatal, the characteristic symptom being respiratory distress.

All those outbreaks were subsequently proved by virus isolation to be Newcastle disease. Strains of virus isolated by the respective authors at Java, Manila, Japan and England were inter-changed and cross-immunity tests were performed which demonstrated the viruses to be indistinguishable immunologically, (Doyle, 1935).

Subsequent reports indicated clearly that Newcastle disease was world-wide in distribution outbreaks being reported by: Crawford (1930) in Ceylon; S. Australia Department of Agriculture (1931) in Victoria; Hudson (1937) in East Africa; Traub (1942) in Germany; Beach (1944) in U.S.A.;

Cernianu and Popovici (1944) in Rumania; Canham, Diesel and Coles (1946) in South Africa; Orr and John (1946) in Malaya; Buck (1947) in Madagascar; Zulinski (1947) in Poland; Walker (1948) in Canada; Isaksson, Kull and Nordberg (1948) in Sweden; Lucam (1948) in France; Daubney and Mansy (1948) in Egypt; Jansen, Kunst, van Dorssen and Berg (1949) in Holland; d'Arces (1949) in Algeria; Blanco (1949) in Spain; Berke and Golem (1949) in Turkey.

Before the true nature of these serious outbreaks of disease was fully realised and their identity with Newcastle disease established a varied and confusing nomenclature was inevitable and as a result synonyms are numerous. These include Phillipine fowl disease (Rodier 1928), Pseudovogelpest (Picard 1928), Korean Poultry disease (Konni, Ochi and Haschimoto 1929), Ranikhet disease (Cooper 1930), Avian Pest (Farinas 1930), Pseudo poultry plague (Johnstone 1931), Madras fowl pest (Kylasamaier 1931), Avian distemper (Haddow 1933), Doyle's disease (Haddow 1935), Chosen disease (Nakamura, Oyama and Wagatsuma 1937), Pneumo-encephalitis, chicken flu, and nine day pneumonia (Beach 1942).

Etiology

The causative agent described by Doyle (1927) is a virus which passes Chamberland L.3, Seitz, and Berkefield filters. Burnet and Ferry (1934)

have shown by filtration methods that Newcastle disease virus (N.D.V.) is 80 to 120 mμ. Bang (1946) has estimated its size at 115 mμ from electron micrographs.

Burnet (1942) has pointed out the close resemblance between Newcastle disease virus and influenza virus regarding their similar particle size, similar haemagglutinating effect on erythrocytes and the similarity in the lesions they produce during their multiplication in the developing chick embryo. He postulated that these viruses may have had a common ancestor but observed that there was no serological relationship.

The N.D.V. is related to the mumps virus. The presence of neutralising and anti-haemagglutinating factors against N.D.V. has been found in the sera of persons convalescent from mumps. (Jungherr et al 1949, Kilham et al 1949, Evans 1951). A similar cross-reaction between the viruses has been demonstrated by the complement fixation test (Jordan and Feller 1950).

This would appear to indicate an antigenic similarity between the viruses. Cross absorption studies by Evans (1951) suggested that the N.D.V. inhibitor represented an "immunological overlap to infection by mumps virus".

The Virus - Morphology:-

The first electron micrographs of N.D.V.

(Bang 1946) demonstrated the virus as filamentous with a large head resembling the comma-shaped bacteriophages of E.coli. Similar descriptions were given by Cunha et al (1947) and Reagan et al (1948). The first group of workers gave a detailed description of the morphology of the virus and they considered its morphology unique and suggested that it showed more clearly than any other virus internal differentiation resembling the analogous structure of living cells.

Bang (1947, 1948 and 1949) has demonstrated that N.D.V. has a spherical shape in water or .8% saline and that at concentrations of saline of 2% or more the virus assumes an elongated form. He has also shown that the virus can revert to the spherical form when returned to water without loss of infectivity.

Reagan et al (1952) claimed that variations in morphology were associated with age of the virus. They contended that in early growth the particles were spherical and that filamentous prolongations developed later. Passage of the virus through such diverse hosts as the mouse, hamster, cave-bat, and green turtle induced little or no change in its morphology. (Reagan et al, 1948, 1950, 1951 and 1953).

Chemical Composition:-

Cunha et al (1947) following concentration of

the virus by ultra-centrifugation gave a chemical analysis of 51.8% carbon, 9.86% nitrogen and .85% phosphorus. They showed the virus to consist of approximately 67% protein, 27% lipids and small amounts of nucleic acid some of which is of the desoxypentose type.

Viability:-

Effect of Exposure under Natural Conditions.

Picard (1928), Dobson (1934) and Hudson (1937) conducted experiments to estimate the period of contamination of pens and cages. Susceptible fowls were exposed in uncleansed contaminated cages. The results indicated that such cages were infective 7 days after contamination but not after 14 days. Jungherr (1948) reported on the stocking of infected cages two weeks after an outbreak and no clinical disease was observed in the exposed fowls. However after 4 - 5 weeks several of the birds showed significant H.I titres to N.D.V. Farinas (1930) demonstrated that infected spleen and liver covered by dry sandy earth was inactive by feeding 12 days later, as was infected crop content after 14 days when mixed with garden soil. On two occasions he failed to infect chickens with drinking water mixed with infected crop contents which had been stored for 4 days.

Effect of Light.

Farinas (1930) exposed diluted virus in Petri dishes to sunlight for ½, 1 and 3 hours and found that even the shortest exposure killed the virus. Burnet and Ferry (1934) demonstrated that N.D.V. was capable of resisting photodynamic inactivation by methylene blue at a concentration of 1/50,000 for 30 minutes. Brandly et al (1946) showed that the virus was readily destroyed by ultra-violet irradiation. Atanasiu and Suoto Patuleia (1952) have confirmed this observation and also noted that the infectivity of the virus decreased much more rapidly than did the haemagglutinating property under this form of treatment. They suggested that the nucleo-protein responsible for virulence may be more sensitive to ultra-violet irradiation than the enzyme responsible for agglutination.

Effect of heat.

Farinas (1930) demonstrated that the virus was inactivated in 30 minutes at 75°C, 62°C and 55°C but that virus held at 50°C or below for 30 minutes remained infective. He also showed the virus to be active after 48 hours exposure at 37°C but not after 72 hours at this temperature. Brandly et al (1946) have shown that the infectivity of 2 strains of N.D.V. were lost after 30 minutes at 60°C and within 45

minutes at 55° C. Asplin (1949) has found that the virus may still be active after a 45 minute exposure to 56° C but that it is inactive after a 60 minute exposure to this temperature. Bushnell and Erwin (1950) assessed the thermal death point at between 58° C and 64° C for a 30 minute exposure.

Effect of Cold.

Doyle (1927) showed that under refrigerated conditions N.D.V. in normal saline remained active for 86 days and in 50% glycerine was active at 197 days but inactive at 259 days. Doyle (1933) referred to the viability of N.D.V. in chilled carcases. He found bone marrow, and muscle from such carcases still infective after 6 months refrigeration. Asplin (1949) has reported that the skin and bone-marrow from plucked and eviscerated carcases of artificially infected birds, kept at 35° F were infective for 98 days and 134 days respectively. Skin and Bone-marrow of unplucked and uneviscerated carcases stored under similar conditions were infective for 160 and 196 days respectively. Doyle (1927), Picard (1928) and Cooper (1931) have reported on the viability of N.D.V. infected material which had been dried and held at refrigeration temperature. The minimum period of activity was 35 days and the maximum 125 days.

Effect of Chemicals.

Doyle (1927) tested the effect of a large number of disinfectants against N.D.V. and found that potassium permanganate would inactivate the virus at 1/5,000 dilution, lysol was effective at 1/1,000 and sodium hydroxide at N/50. The remaining disinfectants tested were either ineffective or effective only at relatively low dilutions. Farinas (1930) showed that both 1% chloroform or 1 to 2% formalin was lethal to the virus in 30 minutes. Johnstone (1933), in connection with the Australian outbreak, quotes .5% sodium hypochlorite in water as the most effective disinfectant tested.

Cunningham (1948) produced results based on a 3 minute exposure of virus to a series of disinfectants. The following agents destroyed the virus in 3 minutes: ethyl alcohol 70%, mercuric chloride 1/1,000, Tr.metaphen undiluted, Tr.zephiran undiluted. Phenol 3%, liq. cresolis saponatus 3%, lysol 1%, Tr. iodine 1%, Sodium hydroxide 2%.

Asplin (1949) also investigated the effects of various disinfectants. He found lysol 2.5% effective in 1 minute and 1% in one hour. Phenol 2.5% lowered the titre in 1 hour and killed in 24 hours. A proprietary hypochlorite 5% lowered the titre in 1 hour and destroyed in 3 hours. Pot.permanganate 9.4% destroyed in 1 hour. The effect of formalin

varied with the temperature: 0.2% at 34 - 35°F inactivated N.D.V. in 10 days, 2% at 65°F inactivated virus in 12 hours and 0.1% at 98°F in 6 hours.

Effect of Antibiotics.

The virus is highly resistant to the action of antibiotics and this property permits the utilisation of antibiotics to suppress bacterial contamination in inocula for diagnostic purposes. This is a considerable improvement in the filtration methods formerly required. Penicillin alone or in combination with Streptomycin is normally used. (Brandly et al 1946, Delaplane 1947, Thompson and Osteen 1948, Beaudette Bivins and Miller 1949, Cunningham 1952).

According to Kohn (1953) when N.D.V. is incubated longer than 3 hours with more than 5,000 units of Penicillin per ml. there is at least 100 fold reduction in its infectivity and up to 2 million EID, 50 are completely inactivated.

Haemagglutination.

The discovery of the haemagglutinating property of influenza viruses by Hirst (1941) and McClelland and Hare (1941) was quickly followed by Burnet (1942) demonstrating that N.D.V. also possessed this property. Electron microscope studies by Heinmets (1948) and Dawson and Elford (1948) have shown that the virus particles attach themselves to erythrocytes and form bridges between the adjacent red cells, resulting in a fine network of erythrocytes being thrown down instead of the normal sedimentation into a button form. The live virus is only attached to the erythrocyte for a short space of time and in the case of N.D.V. elution from the red cell takes place at room temperature. The virus is unharmed by its attachment to the erythrocyte and its haemagglutinating, infectivity, and cell sensitising properties are unaltered. On the other hand the erythrocyte which has been attacked by the virus is permanently altered and a second application of fresh virus to such sensitised erythrocytes will not induce Haemagglutination.

Burnet McCrae and Stone (1946) have arranged the haemagglutinating viruses in order of their activity in damaging red cells thus: Mumps, N.D.V. influenza A, Influenza B and Swine Influenza viruses. Erythrocytes treated by a given virus in the list are agglutinable by the succeeding viruses but not by

the preceding ones. The surface configuration of treated cells is also changed and they become readily agglutinated by a component present in many normal sera. The erythrocytes are then referred to as pan-agglutinable and combine with the 'T' agglutinin of human sera. This phenomenon was first described by Thomson (1926). Burnet et al (1946) found that a filtrate of Clostridium welchii type A culture and also a soluble enzyme (R.D.E.) in filtrates of Vibrio cholera produced a similar effect to viral sensitisation treatment.

Both McClelland and Hare (1941) and Hirst (1942) drew attention to the inhibitory action of immune ferret or human sera against haemagglutination by the influenza virus. Hirst (1942) described a close relationship between haemagglutination-inhibition and serum neutralisation results. With regard to N.D.V. the haemagglutination-inhibition test has now become one of the standard procedures used in diagnosis of this disease.

Burnet and Anderson (1946) have described how human erythrocytes treated with fluid preparations of N.D.V. developed a new antigenic character which allowed them to be agglutinated to high titre either by experimental N.D. immune serum or by most sera from recent cases of infectious mononucleosis in man. The changed character of the erythrocytes was due to the absorption to their surface of an agent other

than the virus.

Beach (1948) has shown that different strains of the virus may vary in their haemagglutinating ability but that this could not be related to variation in virulence. Bang and Foard (1952) pointed out that with the mild Blacksburg strain haemagglutination is so transient that tests must be read within 30 minutes as they may be completely negative at 60 minutes. In a study of 25 strains obtained from various parts of the world, Winslow et al (1950) found that 6 agglutinated the red cells of the horse, cow, goat and sheep. 13 agglutinated those of the first 3 species only and 6 failed to agglutinate any. The erythrocytes of certain individuals in each species were more susceptible than others but generally the behaviour of the erythrocytes of any given individual were constant.

Haemolysis

Investigation into the haemolytic property of N.D.V. has been undertaken by Burnet (1949), Burnet and Lind (1950) and Burnet (1950). In these papers N.D.V. was described as being haemolytic for the erythrocytes of several species of mammals and birds. It was stated that allantoic fluid virus was only weakly haemolytic and paradoxically that it was more actively haemolytic when diluted than when used undiluted.

Virus precipitated from allantoic fluid with methanol and dialysed free from this substance was much more actively haemolytic than the original. Haemolytic activity was lost by heating at 54° C for 30 minutes, a treatment which left intact the haemagglutinating and inhibitor-destroying properties of the virus. At temperatures below 24°C with human cells and 28°C with fowl cells the speed of haemolysis fell very rapidly and at 18 °C or below it was inappreciable.

With an actively haemolytic virus and a high virus/cell ratio haemolysis may be complete, but in general haemolysis proceeded only to a certain level and then virtually ceased. The final level is determined by the relative concentrations of virus and cells, the strain of virus, and the degree to which cell receptors had been modified by previous treatment. Cells from which receptors were removed by R.D.E. or other viruses were completely resistant to haemolysis. The initiation of haemolysis took place only at temperatures above a critical point but once initiated haemolysis proceeded at a lower temperature. The speed with which the initiating reaction occurs was the main factor determining the shape of the haemolytic curve.

Calcium had an inhibitory effect on haemolysis much more evident with fowl cells than with human cells and with allantoic fluid virus than with

Methanol precipitated virus. Haemolysis was also inhibited by immune sera totitres corresponding closely to the antihaemagglutinating titres of the sera concerned. Virus firmly attached to lysed fowl cells haemolysed added normal red cells.

In offering an explanation of the above phenomena Burnet postulated that N.D.V. had 2 characteristic modes of action on erythrocytes. Type I action corresponded to that of most influenza viruses, i.e. reversible absorption associated with enzymic destruction of the receptors and elution. This was shown to varying extent under all the conditions which allowed recognisable interaction between virus and cell surface and was the only type shown below 25°C or by the virus after heat treatment between 54°C and 58°C. Type II action - irreversible union of virus to cell receptors was observed only with active unheated virus, acting on cells at a temperature above 24°C in the case of human cells or 28°C in the case of fowl cells. The same conditions were necessary for the manifestation of 3 other actions by N.D.V. on erythrocytes, viz. haemolysis, reduction in electrophoretic mobility, and extension of haemagglutinating titres at 37 C.

Kahnke (1951) has evolved a haemolytic inhibition test on similar principles to the haemagglutination inhibition test. He observed that the greater the concentration of N.D.V. the greater the

haemolysis produced on fowl erythrocytes and the greater the amount of immune serum added to the virus the greater the inhibition of haemolysis. Based on these findings a laboratory test for N.D.V. antibodies was presented and the results of this test correlate with those of the H.I. Test.

Studies of partially purified preparations of the large and small components (L. and S.) of the virus by Granoff Liu and Henle (1950) suggest that the L. component readily causes haemolysis and that the S. component is non-haemolytic. Granoff and Henle (1954, I and II) have shown that alternate freezing and thawing, sonic vibration, osmotic shock or precipitation by methanol of the virus increased its haemolytic activity.

Tissue Cultivation.

Bankowski and Boynton (1948) have succeeded in cultivating N.D.V. in a modification of Simms-Sanders medium in which bovine or fowl serum replaced pig serum and chick embryo liver or heart replaced pig bone-marrow. N.D.V. was successfully carried through ten serial passages in this medium. Virus propagated in the bovine serum medium had a tendency to lose virulence for chickens. The embryo infective titres of tissue culture virus fluctuated considerably, but titres of 10^{-5} or 10^{-6} were obtained with some degree of regularity. Bankowski (1950) described further studies on in vitro cultivation

of N.D.V. and showed that after the 41st to 50th passage it failed to produce death in chickens 22 to 81 days old. It was however still lethal for chick embryos and 2 day old chicks. This passaged virus induced a satisfactory haemagglutinating-inhibition response and chickens vaccinated with this virus withstood challenge by virulent virus.

Day et al (1953) found that after the 20th passage in a medium containing whole chick embryo N.D.V. lost some of its pathogenicity for baby chicks when inoculated intra-muscularly but when grown in a medium containing chick embryonic skin it was extremely pathogenic for baby chicks when inoculated by the same route.

The development of the N.D.V. in epithelial and fibroblast cells in tissue culture was described by Bang (1953). Long delicate projections, apparently containing virus particles, develop from the surface of the infected epithelial cells before morphological changes are seen in its substance. The fibroblasts are usually destroyed releasing scattered spherical virus particles. Pereira and Gompels (1954) have succeeded in growing N.D.V. in roller-tube cultures of chick embryo epithelial and mesenchymal tissues. Virus activity was demonstrated by the cytopathogenic effect by the Ph differential test and by haemagglutinin and egg infectivity titrations.

Cultivation in Eggs.

Burnet and Ferry (1934) were the first to induce Newcastle disease infection of chicken embryos and to describe it. They reported on the inoculation of N.D.V. on to the chorio-allantoic membrane of 9-10 day old developing chick embryos and found that death of the embryo usually occurred within 30-48 hours of inoculation with characteristic lesions. These included thickening of the chorio allantois and some degree of cloudiness due to cellular infiltration with minute fine grey foci or of plaque-like thickenings with satellite foci. Petechiae of the chorio-allantoic membrane were also noted and also petechiae on the embryo itself particularly along the neck and the ventral aspect of the body. Histological examination of the lesions showed the essential feature to be patchy ectodermal proliferation associated with gross vacuolation of the epithelial cells and eventual necrosis.

Jungherr et al (1946) confirmed these findings and in addition drew attention to the gross congestion and haemorrhagic involvement of the yolk sac. They also described distinct petechiae of the embryo commonly found with strain 11914 (California) and less frequently with the other strains. Congestion of the feet and skin varied from slight to moderate, and there was complete absence of haemorrhage in the skeletal muscles. After 45 hours incubation embryos inoculated with N.D.V. showed defective development

of the lens and auditory sac in 90% of cases (Blattner and Williamson 1951).

Bang (1953) studied the development of N.D.V. in cells of the chorio-allantois by the use of thin sections in the electron microscope. Three strains of N.D.V. representing a wide range in virulence were studied. It appeared that the virulent strain rapidly destroyed the host cytoplasm both at the surface and deep within the cell where apparent virus particles were found. The less virulent strains destroyed few cells but caused hyperplasia and seemed to be present in increasing amount in the surface layer of cells. As in tissue culture this strain was manifest by a profusion of long filaments which seemed to be modified microvilli. These ballooned at the top and broke open or broke off in clusters of particles. The "avirulent" form of the virus can thus be released from the cell without causing cell destruction. Forms similar to those seen in tissue culture and in sections were found in infected allantoic fluid and Bang suggested that the infectious virus particles thus released may not be free from host cytoplasm.

According to Kilham Morgan and Wyckoff (1951) electron micrographs of sections of infected egg membranes showed thickening of the superficial membrane of infected cells followed by vacuolation. Particles on the surface of the epithelial cells appeared to be morphologically similar to N.D.V.

Epizootiology:-

The earlier reports on this subject have been reviewed by Beaudette (1943). They suggest that spread of Newcastle disease has occurred mainly by means of infected exudates, excreta and through the medium of offal from infected birds. Traffic in live birds is suggested as a major factor in dissemination of the disease.

The part played by infected eggs in transmission of Newcastle disease has received considerable attention. Beaudette (1949) assessed the proportion of infected eggs laid during the depressed laying period early in the disease to be as high as 33%. Hofstad (1949) confirmed this finding but was unable to demonstrate N.D.V. in eggs laid during the return to full production after an outbreak nor was he able to find any signs of Newcastle disease infection in hatched chickens. The only evidence of direct egg transmission is a report by De Lay (1947) who isolated N.D.V. from the yolk sac of 4 day old chicks hatched from eggs from an affected flock. The value of this report would appear doubtful in view of the experimental evidence of Burnet and Ferry (1934), Jungherr et al (1946), Doll Wallace and McCallum (1950).

The live infected bird is an obvious source of infection and Walker and Powell (1950) succeeded in isolating virus from the discharges over a period

of 21 days following artificial infection. They also record that healthy hens in contact with a cock infected 6 months previously developed the disease after 5 weeks exposure.

Infection may persist in poultry houses for varying periods. Dobson (1939) recorded a case where there was a recurrence of disease on restocking premises 7 weeks after slaughter. Levin et al (1950) introduced susceptible chicks to 18 farms on which the fowls had recovered from Newcastle disease. Disease developed on 3 farms, 2 of which showed evidence of disease when the birds were introduced.

Kraneveld and Mansjoer (1950) found that the addition of infected faecal material to the water of open drains at a concentration of 1/1000 caused infection and death of chickens after 5 days.

De Lay, De Ome and Bankowski (1948) have demonstrated that the air in poultry houses containing infected birds contains the virus, and that chickens could be infected while suspended in a cage 4½ ft. above the floor.

Outbreaks have frequently resulted from the importation of infected poultry carcases from countries where the disease is endemic. When the disease was introduced to Great Britain in 1947 the first outbreak was confirmed 13 days following the release of uneviscerated poultry carcases imported from Hungary where the disease was then

widespread (Gordon and Asplin 1947). In 33% of the first 540 outbreaks which followed there was a history of access to swill or butcher's offal and in 42% the infection was attributed to traffic in live poultry through dealers' sales, auction marts and pet shops; local spread through mixing and straying of poultry or visits and handling of birds by dealers, infected clothing and crates represented only 15% (Gordon, Reid and Asplin 1948).

Free flying birds have been suggested as vectors by Farinas (1930) and Cooper (1931) although no actual virus isolations were effected. The more recent literature has shown that the virus has been isolated from the following birds in association with outbreaks: Pigeon (Iyer 1939), starling (Gillespie, Kessel and Fabricant 1950), Great Horned Owl, (Ingalls, Vesper and Mahoney 1951), parakeet, (Zuydan 1952), sparrow, (Gustafson and Moses 1953), gannet, (Wilson 1950), osprey, (Zuydam 1952) and cormorant, (Blaxland 1951).

Malbrant (1942) from his observations of Newcastle disease in the Middle Congo considered that the infection had originatd in the Belgian Congo. Natural infection appeared to have occurred as a result of faecal contamination in most cases. However many flocks appeared to have contracted infection without any obvious contact and in these cases sparrows or pigeons were held responsible.

Spread of infection in the Palestine outbreaks was attributed by Komarov (1940) to direct contact between neighbouring flocks. The source of initial outbreaks remained obscure but imported fowls were suspected. Infected carcases of dead birds discarded along the main highways by truck drivers were suggested as sources of new foci of infection.

Regarding the Newcastle disease epizootic in Italy the origin is again obscure. Bianchi (1941) stressed the irregularities in spread particularly regarding temporal and geographical factors. Viamello (1941) observed that transmission of the disease occurred with great facility during cohabitation of infected and uninfected stock. The high diffusibility of the virus was held responsible for the inadequacy of the hygienic and isolation control measures.

Wagener (1941) ascribed the introduction of Newcastle disease into Germany to the entrance of wild and domestic birds from Italy to Hanover. Outbreaks in Bavaria, (Beck, 1942) and in Oldenburg, (Steffens, 1942) were associated with the introduction of pheasants from Hungary to these districts for the improvement of the local stocks. Further outbreaks at Dusseldorf and Arnsberg coincided with the arrival of Hungarian market poultry (Koser, 1942). In these cases the transfer of infection to rural stocks was definitely traced to the offal from market poultry. Initially the disease spread slowly by

the normal contacts between flocks but following widespread release of emergency slaughtered poultry the disease assumed epizootic proportions.

In the United States the evidence regarding introduction or origin of the disease is unknown. It is believed that a mild form of Newcastle disease originated in California about the middle thirties. Beach (1944) first demonstrated that Newcastle disease existed in the U.S.A. and was in fact widely distributed. Transmission by contact was frequently observed (Stover 1942, Beach 1942) before the etiology was established. Stover (1942) failed to observe the transmission of infection between birds in adjacent cages and assumed that infection normally occurred by ingestion of contaminated foodstuffs. Nevertheless he used the trachae and lungs from infected birds for successful passage of the virus by respiratory instillation.

There is no evidence that ectoparasites play any part in transmission of the disease. Ticks (Argas persicus) were found feeding on infected fowls but when applied to healthy fowls failed to transmit infection, (Komarov 1940). Susceptible fowl inoculated with an emulsion of such ticks developed the disease. Hofstad (1949) was unable to transmit the disease with mites (Lyponyssus sylviarum). Bolin (1948), however, found the virus present in lice taken from hens 35 days after subcutaneous injection with the virus.

The use of commercial fowl pox and laryngo tracheitis vaccines contaminated with Newcastle disease virus has been responsible for the introduction of the disease into poultry and turkey flocks (Zargar and Pomeroy, 1950).

Species Susceptibility and Clinical manifestations

The domestic fowl must be regarded as the most susceptible host to N.D.V. infection. There is considerable variation in the symptoms of the disease depending on the type of virus involved and on the susceptibility of the affected flock. Chicks were most susceptible resistance grew with age and some older birds have proved refractory to both natural and experimental infection (Dobson, 1939).

Doyle (1927 and 1933) made the following observations. The incubation period varied from 4 to 11 days with 5 days as the average. The febrile reaction was first noted, a considerable rise in temperature taking place before any other symptom developed. The temperature rose to its peak at the 7th day following infection and then fell rapidly to normal or subnormal at death. About the 5th day anorexia developed, the respiratory rate was increased and a crouching attitude was assumed by the subject with eyes half-closed presenting a sleepy appearance. A watery yellowish-white diarrhoea was observed with a characteristic nauseating odour. Peculiar long gasping inhalations were noted in about 70% of affected birds. In the majority of cases a thick mucous discharge was observed from the nostrils and a variable amount of frothy exudate hung from the beak Cyanosis of the comb and wattles developed and death

supervened about 48 hours after the onset of symptoms.

In the sub-acute cases Doyle described nervous symptoms such as twitching of the head, and lameness or paralysis of the limbs.

The mortality figure varied from 95 to 100%.

North American strains:-

With the discovery by Beach (1944) of the existence of Newcastle disease in North America there was presented a completely different clinical picture. Jungherr et al (1946) have described the clinical manifestations. In the naturally occurring cases in the U.S.A. the hyperpyrexia associated with the European outbreaks was not found. The cardinal symptoms were coryza and nervous disorders. Most striking were the early signs of incoordination, or slight tremor, or without these forerunners, the sudden development of rapid rhythmic twitchings of one or more extremities or of the head and beak. In these individuals leg and wing paresis or other aberrant attitudes, including intermittent or persistent torticollis, opisthotonos, or emprosthotonos were relatively common. In baby chicks and growing chickens there were generally symptoms of hoarse chirping, coughing and sneezing. Appetite was affected and the birds appeared cold and huddled in groups. Jungherr et al (1946) also drew attention to the fact that the gasping for air symptom associated with the natural disease was infrequently seen after experimental parenteral infection. After respiratory tract instillation or passage by contact this symptom was common. This points to the respiratory channel of infection as the common mode of transmission

in the natural disease. Jungherr (1948) drew attention to the diminished egg production and the production of soft-shelled eggs which were common features of the disease. Knox (1950) studied the effect on egg production of an outbreak that occurred in 1947-48 and has shown that the disease had a 12 week effect on production with an egg loss of 26 to 43%. The average egg weights were also below normal.

The mortality varies from 0 to 50% (Beach 1948). The age of the birds and the incidence of intercurrent disease - avitaminosis, malnutrition, superimposed infection, tending to increase the mortality.

The earliest reports (Kraneveld 1926, Crawford 1930, Albiston and Gorrie 1942) have confirmed the turkey as being highly susceptible. The symptoms produced being similar to those described in the fowl. Hurt (1948) pointed out that although Newcastle disease spread more slowly in turkeys than in fowls the mortality in the former reached 40 to 60%.

There are several records of exposure of ducks and geese to both natural and experimental infection (Doyle 1927, Crawford 1930, and Dobson 1939) but both have proved highly resistant. Iyer (1945) failed to demonstrate antibodies in the sera of inoculated ducks and Asplin (1947) reported that although H.I. antibodies developed in ducks

which had been in contact with infected fowls, the response was not so regular nor the titre so high as in chickens.

Deaths in Guinea-fowl have been reported in outbreaks affecting fowls by Farinas (1930) Crawford (1930) and Hudson (1937).

Pheasants were highly susceptible, (Levine, Fabricant and Mitchell 1947). The symptoms being tremors, inco-ordination, and a high death rate.

The disease was also recorded in the peacock (Jansen and Kunst 1952) and isolation of virus from naturally affected birds has taken place in the following cases: Gannet (Wilson 1950), Starling (Gillespie, Kessel and Fabricant 1950), Cormorant (Blaxland 1951) and Osprey (Zuydam 1952).

No case of Newcastle disease occurring naturally in any mammal has been proved except in man. Burnet (1943) described an infection caused by the accidental squirting of infected allantoic fluid into the eye. The next morning there was acute conjunctivitis and the right pre-auricular gland was swollen and tender. There was headache, chilliness and discomfort. The following day there was improvement. The condition subsided in 2 weeks. The virus was recovered by egg inoculation and antibody was

demonstrated in the convalescent serum by H.I. and serum neutralisation tests. Two further laboratory infections in humans have been described by Anderson (1946) both being cases of mild bilateral conjunctivitis with regional adenitis. In Palestine cases of conjunctivitis due to Newcastle disease have been described both in laboratory workers and amongst individuals preparing table poultry (Shimkin 1946, and Yatom 1946). As mumps convalescent serum possess antibodies inhibitory to N.D.V. reliance can only be placed on reports where the virus has actually been isolated (Evans 1951).

In a more recent report Moolten and Clark (1952) claimed that N.D.V. is responsible for a proportion of the cases of idiopathic anaemia in man.

Experimental Hosts:-

Artificial infection of most wild birds can be accomplished readily but the degree of susceptibility appeared to vary from species to species and was also dependent on the strain of virus used (Pagnini 1942, Kaschula 1950, and Collier and Dinger 1950).

Doyle (1927) reported the pigeon susceptible and at the time this constituted a valuable method of differentiating N.D.V. from Fowl plague virus. The symptoms were similar to those of domestic fowls, viz. drooping of the wings, paralysis of the legs, sleepy appearance and death in 5 to 6 days.

Beaudette (1943, 1949, 1950, 1951, 1953) in his reviews discussed the extensive literature on the experimental infection of many species of birds, including grouse, pheasants, quail, partridges, sparrows, starlings, pigeons, turtle doves etc.

Laboratory animals can also be infected artificially. Mackenzie and Findlay (1937) showed that the ferret and fitch were highly susceptible to N.D.V. infection by the intracerebral, intraperitoneal, or intranasal routes producing in them symptoms of an ascending myelitis. P.M. examination showed bronchopneumonia and haemorrhagic lesions of the stomach, liver and kidneys. Findlay and

Mackenzie (1937) also demonstrated infection of mice by intracerebral passage. Burnet (1942) reported that mice inoculated intracerebrally with N.D.V. died with similar lesions to those produced by the influenza virus. Hanson, Upton and Brandly (1951) have shown that different strains of virus installed into the nares of mice 21 to 30 days old varied in their pneumopathic activity. Some caused extensive pneumonia and death within 96 hours while others produced no obvious symptoms. Ginsberg (1951) has shown that mice could be infected intranasally and that extensive pulmonary consolidation was produced by the N.D.V. particles in the absence of virus multiplication. Liu and Bang (1952) showed that older mice were more resistant to the neurotropic effect of the virus and less resistant to the pneumotropic effect and that while some strains of virus can be passaged successfully by intracerebral inoculation of adult mice continued passage of others is only possible in the brains of unweaned mice. Kilham, Loomis and Peers (1952) using intra-cerebral passage of N.D.V. showed that a definite encephalitis of a progressive type was produced which was usually fatal in 7-10 days. The lesions appeared concentrated in the gyrus hippocampi and the frontal and temporal lobes. The brain was the only organ in which lesions were

observed.

Zuydam (1951) examined wild rats caught on infected farms but could not isolate virus. Wild rats fed on infected material, excreted the virus for 1-2 days but showed no symptoms. Asplin (1949) has shown similar results with laboratory rats.

Verge, Placide and Santucci (1949) attempted intracerebral transmission in the rabbit unsuccessfully.

Wenner and Lash (1949) succeeded in causing a meningo-encephalitis in monkeys following intracerebral inoculation. Collier, Polak and Verhaert (1950) confirmed this finding and described lesions closely resembling those of Japanese B encephalitis.

Reagan et al (1947 and 1949) described the effect of intracerebral inoculation of N.D.V. into the hamster. Approximately 2/3rds of hamsters so inoculated showed symptoms of irritability malaise and finally death. By repeated passage the incubation period for the hamster was cut from 4 days to 12 hours and after the 300th passage infection was possible by the intradermal route.

Pathology.

Doyle (1927) described the lesions he observed as those of the organic changes due to fever. He dres attention to the petechiae so characteristic of this disease, the chief sites being on the gizzard

fat, the pericardial sac, the heart muscle, and the lining membrane of the proventriculus. He also described congestion of the trachae, slight congestion of the lungs in some cases and catarrhal enteritis.

A detailed description of the post-mortem appearances was given by Albiston and Gorrie (1942) referring to the acute Victorian outbreak of 1931. The predominant lesions were haemorrhagic extravasations in various parts of the body. The most constant and typical lesion was submucous petechiae or ecchymosis in the proventriculus. The amount of haemorrhage in this organ varied from a few areas of extravasation to a marked haemorrhage involving the whole of the proventricular mucosa. This was frequently though not invariably accompanied by a varying amount of submucous petechiation in the intestine, usually well-marked in the duodenum. Actual haemorrhage into the lumen of the bowel was noted in a number of instances. The gizzard fat and the peritoneal covering of this organ were frequently injected, while the peritoneal surface of the ileo-caecal regions showed haemorrhagic changes. Large haemorrhagic blotches were sometimes found in the walls of the crop and oesophagus. More rarely haemorrhages were found on the skin, subcutaneous tissue,

muscles, epicardium and on the visceral surface of the sternum. A clear watery oedema of the peritracheal tissues and the subcutaneous tissues of the throat was sometimes present. There was generally an accumulation of stringy mucous in the mouth, pharynx, and cleft of the palate. In some cases there was an excess of clear, yellowish pericardial fluid, coagulating on exposure to air. The only pathological condition found in the trachea was injection of the mucosa.

A striking contrast to this picture was presented by Beach (1944) in his description of post-mortem lesions of Newcastle disease in California. The gross lesions observed were a mucous exudate in the trachea and in most cases cloudiness of the membranes which form the air sacs and mesentery. Beach (1944) also described that following artificial transmission of the disease the characteristic European post-mortem picture might appear with its extensive haemorrhagic lesions. In a report on the differential pathology of Newcastle disease Jungherr et al (1946) described the essential process in the lungs as proliferative in character causing an interstitial pneumonia and a primary encephalomyelitis.

Immunity.

Congenital Immunity:-

Brandly, Jones and Jungherr (1946) have shown

that chicks hatched from eggs laid by immune hens possess a varying degree of transient passive immunity, a high serum antibody titre being maintained during the first 2 weeks of life, after which it declined rapidly. At the time of hatching the antibody titre of such chicks is almost identical with that of their dams but the titre drops to an insignificant level by the end of the 2nd week. This progressive fall in passive immunity was confirmed by Alberts and Miller (1950) who challenged chicks hatched from eggs of vaccinated dams. Of the challenged chicks 91% of 1 day old chicks survived, 87.4% of 7 day old, 77.2% of 14 day old, 55.3% of 21 day old and 35% of 28 day old. This phenomenon has been considered to be of practical importance as this passive immunity may interfere with the development of active immunity following vaccination of young chicks, (Brandly et al 1946, Beaudette and Bivins 1953). The interference effect is greatest when vaccine is administered by the intra-muscular route, (Bornstein et al 1952) and a better response is obtained by the intranasal or intra-ocular routes (Heitchner 1950, Doll McCollum and Wallace 1950).

Acquired Immunity:-

Mitchell and Walker (1951) suggested that the

use of hyper-immune serum to induce passive immunity might be justified in valuable flocks, but this does not appear to have been practised on a commercial scale.

Active immunity can be produced by exposure of birds to dead vaccine, living vaccine or following recovery from the disease.

Dead vaccines are mainly produced by growing virulent strains of virus in eggs and inactivating the harvested product. Doyle and Wright (1950) used crystal violet ethylene glycol as an inactivator and their product was safe and stored well. Immunity was established at the 7th day and persisted for at least 12 months. Mitchell, Walker and Moyniham (1952) reported on a vaccine consisting of harvested egg fluids treated with formalin and an adjuvant of mineral oil and falba. Over 90% of vaccinated birds were solidly immune 6 months after vaccination.

Following the report by Iyer and Dobson (1940) on the attenuation of the Herts strain of N.D.V. by serial passage in eggs and its successful use as a vaccine, egg propagated vaccines have been extensively employed.

Strains of varying virulence were used from the relatively inocuous Blacksburg or B1 strain, Hitchner and Johnson (1948) administered either

by intranasal or intra-ocular instillation to the more virulent types administered intra-muscularly by the "stab" method, (Van Roekel, Sperling, Bullis and Olesiuk, 1948).

White and Appleton (1953) have found evidence of immunity to respiratory infection within 48 hours of vaccination but resistance to intra-muscular challenge was delayed for 8 days, suggesting that primarily there develops a cell-block or interference phenomenon, later followed by the production of specific neutralising antibodies.

Antibodies inhibitory to the haemagglutinating action of N.D.V. (Lush 1943) and virus neutralising antibodies (Doyle 1927) are present in the blood of fowls which have been exposed to the virus either naturally or artificially.

Beach (1944) has pointed out the great disparity between the Haemagglutination Inhibition (H.I) titre and the virus neutralising titre of the same sera. Brandly et al (1947) reported close correlation between the two antibodies during the ascending phase of immunity. During the descending phase of immunity the haemagglutination-inhibitor disappeared first, thus providing an explanation for the resistance of some birds which are negative to the H.I. test. Hanson, Winslow, Brandly and Upton (1950) confirmed the greater

persistence of the virus neutralising titre and suggested that haemagglutination-inhibition and virus neutralisation were the function of separate antibodies. Schmittle (1953), by low temperature ethanol fractionation of immune serum has isolated globulin fractions rich in both activities which were biochemically different. Osteen and Anderson (1948) and Fabricant (1949) both found that the haemagglutination-inhibitors appeared first. According to the latter the H.I. titre was positive 2 days before to 5 days after the appearance of symptoms, and birds kept for 23 months remained positive.

Control Measures.

Control of Newcastle disease is effected in Great Britain by means of the Fowl Pest Order (1936) and its subsequent amendments. This order requires an owner to report any suspicion of the disease to the police who in turn notify the local Divisional Veterinary Officer. An immediate veterinary investigation is carried out, the findings being based on symptoms, post-mortem examinations and examination of blood samples by the H.I. test. In the event of a positive diagnosis the entire stock of poultry are valued and slaughtered, compensation being paid to the owner at current market values. Disinfection of the premises is

undertaken and restocking of the premises permitted 6 weeks after slaughter.

In most countries where a compulsory eradication scheme is not force, control is attempted by vaccination. Vaccination on a national scale is extremely costly. Pomeroy and Brandly (1953) have estimated that an adequate vaccination programme for the U.S.A. would involve dealing with 300 to 400 million chicks per annum. Brandly et al (1946) suggested that the most satisfactory vaccine policy would be to vaccinate initially with a dead vaccine and 7 to 10 days later with a live virus. At the present time in the U.S.A. the control of Newcastle disease is left entirely to the individual stock owner.

CHAPTER II.

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Virus.

The following viruses have been used in the present investigation.

Newcastle Disease Virus:

1. Hertfordshire Strain. This strain of high virulence was isolated by Doyle during one of the first outbreaks in England.

2. California Strain. This strain of medium virulence was isolated in California, U.S.A.

3. Massachusetts Strain. This strain of medium virulence was isolated in Massachusetts, U.S.A.

4. Blacksburg (B1) Strain. The origin of this strain was obscure. It was obtained by Hitchner and Johnson (1948) from Beaudette of the New Jersey Agricultural Research Station, labelled as a strain of infectious bronchitis virus (71st passage) and was subsequently shown to be a N.D.V. strain of low virulence. It has been successfully used as a live vaccine in the U.S.A.

5. Hebrides Strain. This strain was isolated by Blaxland (1951) from material obtained by the author from a cormorant shot in the vicinity of an outbreak in the island of Harris in 1949. It was in the 2nd allantoic passage when received in this laboratory.

6. Lasswade Strain. This strain was originally a Herts strain which had lost some degree of virulence for adult fowls. This Herts variant was passaged through a captive cormorant and re-isolated.

7. Victoria Strain. This strain was isolated by Albiston and Gorrie (1942) in 1931 from the acute outbreak of Newcastle disease in Australia.

8. Twiss Strain. This strain was isolated by Mitchell and Walker (1948) from an acute outbreak in Canada. When received in this laboratory it had undergone 3 allantoic passages.

Strains 1 to 5 were obtained from the Veterinary laboratory of the Ministry of Agriculture and Fisheries, Weybridge. Strain 6 was isolated in the course of this study. Strain 7 was obtained from the Eliza Hall Institute, Melbourne and Strain 8 from the veterinary laboratory of the Canadian Department of Agriculture. No further information is available regarding the number of allantoic passages undergone by these strains before receipt but during the course of this work each strain has been passaged 12 to 15 times in eggs.

Supply and Management of Eggs.

Fertile Brown Leghorn eggs supplied by the Department of Genetics, University of Edinburgh, were used in the present study. The general

management of the eggs before inoculation was according to the methods described by Beveridge and Burnet (1946).

Allantoic Inoculation.

The technique used for the inoculation of eggs was essentially that described by Burnet and Ferry (1934) with the modifications that the inoculum was introduced directly into the allantoic cavity by means of a tuberculin syringe instead of being deposited on the chorio-allantoic membrane and incubation was carried out at 37°C instead of 39°C. After death of the embryos the eggs were chilled at 4°C for 2 to 16 hours to facilitate the collection of allantoic fluid free from blood.

Preparation of virus pools and storage.

For the preparation of a pool of any virus strain a large batch of eggs was inoculated by the allantoic route in doses of 0.1 ml. The usual dilution of inocula was 10^{-3}. After harvesting, the individual egg fluids were tested for the presence of virus and for sterility and the most satisfactory fluids were pooled. After a light spin in the centrifuge to remove any cell debris the supernatant was placed in screw-capped glass vials and stored at -36°C.

Collection of Erythrocytes.

Erythrocytes from man and animals have been used for haemagglutination, haemolytic and other

tests.

Human Erythrocytes.

A regular supply of these was obtained from the blood transfusion service in the Royal Infirmary, Edinburgh.

Animal Erythrocytes.

These were obtained either by vein or cardiac puncture of the appropriate animal, the blood being collected in citrated saline to prevent clotting. As far as possible the cells from the same animal were used in any particular experiment and in no case were the erythrocytes from different individuals pooled for routine use.

Preparation of Sera.

Specific sera for each strain of virus were obtained from both rabbits and fowls.

Rabbit Sera: Five rabbits were selected and inoculated intra-venously with 1 cc. of virus in the form of infected allantoic fluid. Five strains of virus were used, viz. Herts, California, Massachusetts, Blacksburg and Lasswade. After 14 days a further 1 cc. of the appropriate strain was given intra-venously to each rabbit and 14 days following the second injection a sample of blood was drawn off each rabbit by cardiac puncture under light ether anaesthesia. The blood was allowed to clot and the serum removed and stored at -36° C.

Fowl Sera: Sixteen adult Rhode Island Red fowls

were obtained and isolated in pairs. Each pair was inoculated intra-muscularly with 1 cc. of one of the 8 virus strains previously described. This resulted in the birds developing Newcastle disease and 4 weeks after inoculation the survivors were bled, and their serum collected and stored at -36 °C. This method resulted in high titre sera being obtained for the following strains of virus: Herts, California, Massachusetts.

The Blacksburg strain did not induce an appreciable immune response when given by this route and a further pair of fowls were isolated and inoculated intra-nasally with 1 cc. of infected allantoic fluid. Four weeks after this inoculation a high titre serum was obtained for this strain.

With the Lasswade, Victoria and Twiss strains the original birds used all succumbed to the infection and a further batch of 3 pairs of fowls were placed in separate isolation premises. These were inoculated intra-muscularly with 1 cc. of infected allantoic fluid which had been exposed to ultra-violet irradiation for a period of 15 minutes. All the birds concerned developed symptoms of Newcastle disease but survived and high titre sera were obtained for these three strains.

All sera were stored at -36°C without the addition of preservatives.

Infectious Mononucleosis Sera.

Eight samples of sera from cases of infectious mononucleosis in man, which had been submitted to the Bacteriology Department, University of Edinburgh for examination were selected. All had proved positive to the Paul Bunnell tests having titres of heterophile antibody of 1 in 20 or over after absorption with a suspension of guinea pig kidney.

The Haemagglutination and Haemagglutination-Inhibition Tests.

The method used in this study is described in a supplement to "The Diagnosis of Newcastle disease" (1946) issued by the U.S. Department of Agriculture. This method is now used extensively in Great Britain for the serological diagnosis of Newcastle disease and is a modification of the procedure evolved by Salk (1944).

Haemagglutination Test. This test is carried out in tubes 10 mm. in diameter. Progressive doubling dilutions of virus up to 1/1024 in .85 per cent saline are prepared. The ingredients .2 ml. virus, .2 ml. saline and .2 ml. of .25% fowl erythrocytes are added and the test read at 15, 30 and 60 minutes. A negative result consists of a compact button of sedimented cells in the middle of the bottom of the tube. Agglutination consists of cells covering the entire bottom of the tube, salmon pink in colour, or a heavier layer of cells aggregates which forms a disc with ragged, serrated edges.

Haemagglutination inhibition (H.I.) Test. Using positive, negative, or suspect sera, this test is set up in exactly the same manner as the haemagglutination test except that the serum to be tested is diluted with .85% saline, heated at 56°C for 30 minutes, and substituted for the saline in the haemagglutination test.

A diagram illustrating these tests is given on page 51.

H.I. Antibody absorption Technique.

Absorption of antibody from the prepared specific sera was attempted by the method of Jensen and Francis (1953) who found this method satisfactory when used to analyse the antigenic structure of influenza virus strains. Its application in this study to N.D.V. was unsuccessful and a new technique for antibody absorption was evolved. This technique was based on the stability of the virus-erythrocyte union at 4°C and will be described in the results chapter (page 125).

TEST COMPONENTS:

Each tube	.2 cc virus .2 cc saline .2 cc .25% chick erythrocytes
Control tube	.4 cc saline .2 cc .25% chick erythrocytes

Virus dilutions: 1 | $\frac{1}{2}$ | $\frac{1}{4}$ | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | 1/1024 | Control (no virus)

Virus titration:

Interpretation Titre of virus 1/512, i.e. the highest dilution which agglutinates the erythrocytes. At this dilution there is one agglutinating unit of virus.

Each tube	.2 cc virus .2 cc normal fowl serum .2 cc .25% chick erythrocytes
Control tube	.2 cc saline .2 cc normal fowl serum .2 cc .25% chick erythrocytes

Normal Serum

Interpretation H.I. Titre = 1 unit x dilution of the serum. All normal sera have a slight inhibitory effect on agglutination. In this case 1 dilution is inhibited and the result of the test is negative.

Each tube	.2 cc virus .2 cc Newcastle disease serum .2 cc .25% chick erythrocytes
Control tube	.2 cc. saline .2 cc. Newcastle disease serum .2 cc .25% chick erythrocytes

N.D. Serum

Interpretation H.I. Titre = 256 units x dilution of serum, i.e. the result of the test is positive.

Serum Haemagglutination Test.

This test is based on the discovery by Burnet and Anderson (1946) that erythrocytes treated with allantoic fluid preparations of N.D.V. develop a new antigenic character which allows them to be agglutinated to high titre either by experimental N.D.V. immune sera or by the sera from most cases of infectious mononucleosis in man.

In this work the method used was that described by Evans (1951) and an important point noted was that the use of fresh allantoic fluid preparations was essential as otherwise the erythrocytes were unstable following treatment.

The test has been used in the comparative study of the 8 strains of N.D.V.

Serum Neutralisation Tests.

To supplement the evidence of antigenic variation obtained by the haemagglutination-inhibition tests in ovo cross-neutralisation tests were conducted with 3 strains of N.D.V. and their homologous or heterologous sera. The technique employed was that described by Cunningham (1952).

Method:

Serial ten-fold dilutions of virus infected allantoic fluid were prepared in phosphate buffer saline and thoroughly mixed, using separate pipettes at each dilution. Equal parts of diluted virus and serum were mixed in separate tubes for each dilution. To compensate for the increased dilution of virus when mixed with the serum each virus dilution was mixed with an equal part of diluent in the same volume as was used for the serum-virus mixtures. The mixtures were incubated at room temperature for 1 hour before injection into the indicator host.

Ten day old embryonated chicken eggs were used as indicator hosts. The amount of inoculum was 0.1 cc. per egg injected into the allantoic cavity and the eggs were incubated at 37°C following inoculation. The criterion of viral activity was mortality of the embryo, but mortality within the

first 24 hours was considered to be due to non-specific cause and was not included in the calculation of mortality rates.

By this method the titre of most strains of N.D.V. can be expected to lie between 10^{-8} and 10^{-9}. The difference between the virus titre and the virus/serum mixture titre was indicated as the LD 50 Neutralisation Index (N.I.). With N.D.V. the LD 50 N.I. of sera from normal chickens is $10^{1.001} \pm 10^{.0262}$ or approximately eleven neutralising doses. Four eggs per dilution of virus are the minimum for the determination of the LD 50 as if fewer eggs are used the serum neutralisation test represents only an approximation of the antibody content of a serum.

The LD 50 was calculated by the method of Reed and Muench. (1938).

Growth Experiments in Eggs:

Comparison of the growth rates as indicated by infectivity haemagglutination, and haemolytic activity of allantoic fluids has been attempted in this study for 8 strains of N.D.V.

Method.

The technique employed was the inoculation of a large batch of eggs with .1 cc. of 10^{-3} dilution of infected allantoic fluid for each strain of virus. The eggs were incubated at 37° C and 3 eggs were removed from the incubator every 3 hours until the 100% death point was reached. Each batch of 3 eggs was harvested and the allantoic fluid so obtained was subjected to tests for infectivity to embryos, haemagglutination and haemolysis.

Infectivity Test:

The harvested allantoic fluid from each batch of 3 eggs was diluted to 10^{-3} and 0.1 cc. inoculated by the allantoic route into 6 10 day old embryonated eggs. The eggs were incubated at 37° C and were checked periodically for deaths. The presence of virus was determined by mortality of the embryos and by the haemagglutinating activity of the harvested allantoic fluid.

Haemagglutination Test:

Using the harvested allantoic fluid from each batch of eggs the haemagglutination test was carried out by the method previously described.

Haemolytic Test:

The allantoic harvests were first subjected to a light clarifying spin and the supernatant was taken and its opacity read in the E.E.L. colorimeter using the blue filter. To 10 cc. of the allantoic fluid was added 0.25 cc. washed, fresh, human group 'O' cells, and this mixture was incubated at 37 C for 2½ hours. The fluid was then spun to deposit the erythrocytes and the supernatant was removed and its opacity again read in the E.E.L. colorimeter. The difference in the two readings gave a figure representing the degree of haemolysis that had occurred.

Electron-microscopy.

Erythrocytes from man and different animals show visible agglutination by the Newcastle Disease Virus and its related viruses in vitro but their direct use in electron microscopy is not possible. Dounce and Lan (1943) have described a method of haemolysing fowl erythrocytes by means of a saponin solution. Using this method Dawson and Elford (1949) have succeeded in obtaining photographs of absorbed virus particles.

In this work the method adopted has been to haemolyse fowl erythrocytes, absorb virus on to them, fix with osmic acid, shadow cast with gold-palladium alloy, and demonstrate the lysed erythrocyte with attached virus in the electron microscope. This method is felt to be superior to the method of Bang (1946, 1947, 1948 and 1949) and Reagan et al (1952), who used high speed centrifugation to concentrate the virus, as it ensures that the object under examination is not a normal constituent deposit, or virus grossly distorted in preparation.

The detail of the method is as follows:-

Preparation of lysed fowl erythrocytes: Ten cc. of fresh defibrinated chicken blood were taken and spun and washed twice with 0.9% sodium chloride. The cells were resuspended to their original volume in 0.9% sodium chloride. To 10 cc. of this

suspension were added .5 cc. of .11 molar phosphate buffer Ph 6.8 to 7.0, containing .03 gm. of saponin (6% saponin). The cells were allowed to lake for 5 minutes and were washed thoroughly 4 to 5 times with 0.9% saline M/33 phosphate buffer 6.7. The washed laked cells were then resuspended in 1.5 cc. 0.9% saline buffer and 1 drop of penicillin/streptomycin mixture was added, containing 10,000 penicillin per cc. and 10,000 microgrammes of streptomycin per cc.

Absorption of virus by laked cells.

To 1 cc. of 10% laked cells (prepared by method of Dawson and Elford) was added 1 cc. of virus in the form of infected allantoic fluid. This mixture was held at 0°C for 30 to 60 minutes. An equal volume of .09% osmic acid was added at 0°C and this mixture held for 10 minutes. The cells were washed four times with distilled water and finally resuspended in distilled water to give a faintly opalescent suspension. A drop of this suspension was placed on a copper grid with a prepared supporting collodion membrane and left for 5 to 10 minutes. The excess suspension was drained off with blotting paper and the grid inspected under the low power light microscope for suitably density of cells which should be approximately 5 cells per grid section. The specimen was then dried in the dessicator.

Shadowcasting:

The grids mounted on a suitable frame were held at a distance of 10 cm. from the point source of the volatiliser. The angle used was either 15° or 30° to the plane of the membrane surface and approximately 1 mgm. of the metal was evaporated for less than a second.

The specimen was then inserted in the electron-microscope for examination. The electron microscope used in this study was the Metropolitan Vickers type E.M.3, and operated at 75 K.W.

PART I. EPIZOOTIOLOGICAL OBSERVATIONS

Epizootiological Observations:

Call nan, Cearc

Is ann an so a tha'n seanchas;
Is e air feadh gach baile
Eadar bodaich is paisdean
'S gu seachd araid aig mnathan,
Is aig nionagan oga
Is iad an comhnaigh ag gearain,
Ged nach canadh iad riums'e,
Is mor an iondrainn a th'aca
Air call nan cearc.

This is the opening stanza of a Gaelic poem written by John Campbell of South Uist in 1898, and is probably the first record of an outbreak of Newcastle disease.

The poem describes an epizootic amongst the poultry of the Western Isles in winter of that year which resulted in the total loss of the domestic fowls in the area. The poet makes the point that ducks were quite unaffected by the disease. He also mentions assistance given to the islanders in the form of fresh stocks of poultry and settings of hatching eggs which were supplied by the Congested Districts Board. Confirmatory evidence of this was obtained in the 2nd and 3rd Reports of the Congested Districts Board to the Secretary of State for Scotland (1899 and 1900). These reports indicate

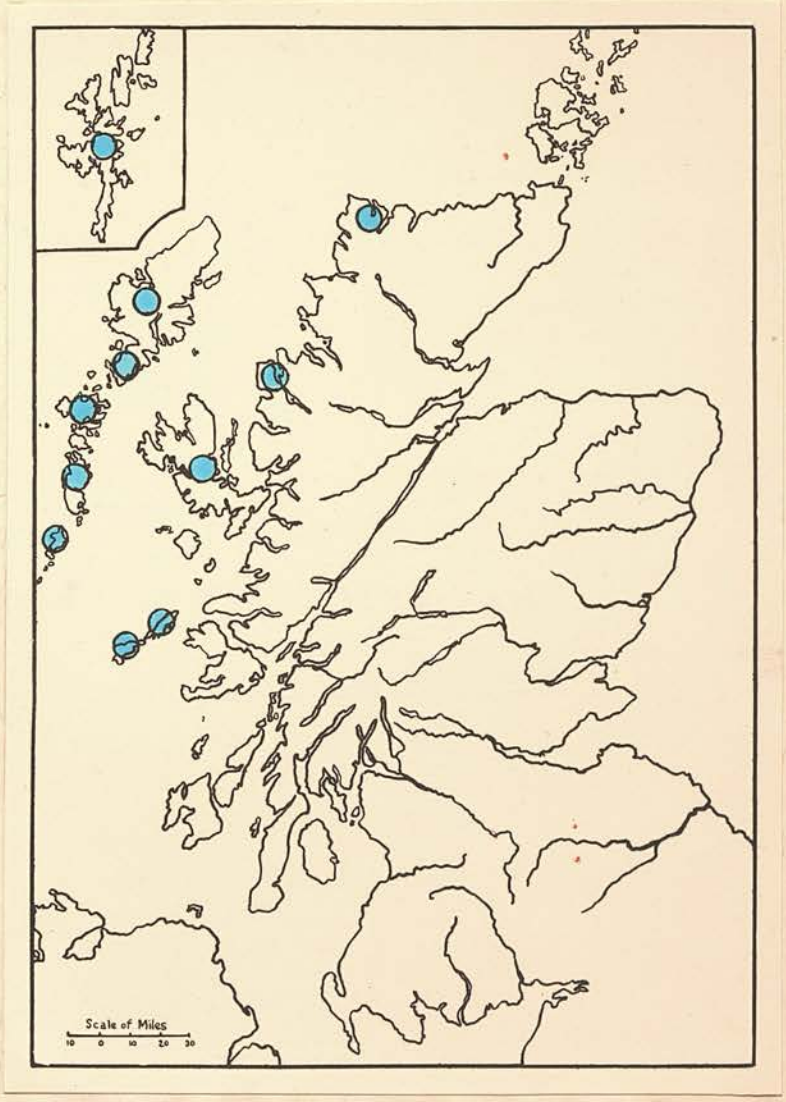
that poultry and hatching eggs were allocated to individuals in the following areas:- Islands of Lewis, Harris, North Uist, South Uist, Barra, Skye, Coll, Tiree and the Shetlands and the mainland coastal areas of Gairloch and Lochbroom (Ross-shire) and Durness and Farr (Sutherlandshire). Unfortunately no reference is made in these reports to any disease having occurred in the poultry, but comparison of the map showing the incidence of outbreaks in the 1949-51 epizootic (Page 63) with the map showing the areas supplied with hatching eggs and fowl by the Congested Districts Board (Page 62) shows an amazing coincidence.

A considerable weight of evidence is also available in the Hebrides amongst many of the people who remember the earlier epizootic and who can describe the symptoms of Newcastle disease very accurately. For instance Alexander Campbell, Glenvargill Farm, Isle of Skye, described the respiratory symptoms of his poultry stock as being "like those of an asthmatic man" and also described the nervous symptoms of inco-ordinated and gyratory movement so characteristic of Newcastle disease in the field.

From 1898 Scotland has enjoyed a long period of freedom from Newcastle disease infection despite the periodic outbreaks which have occurred in

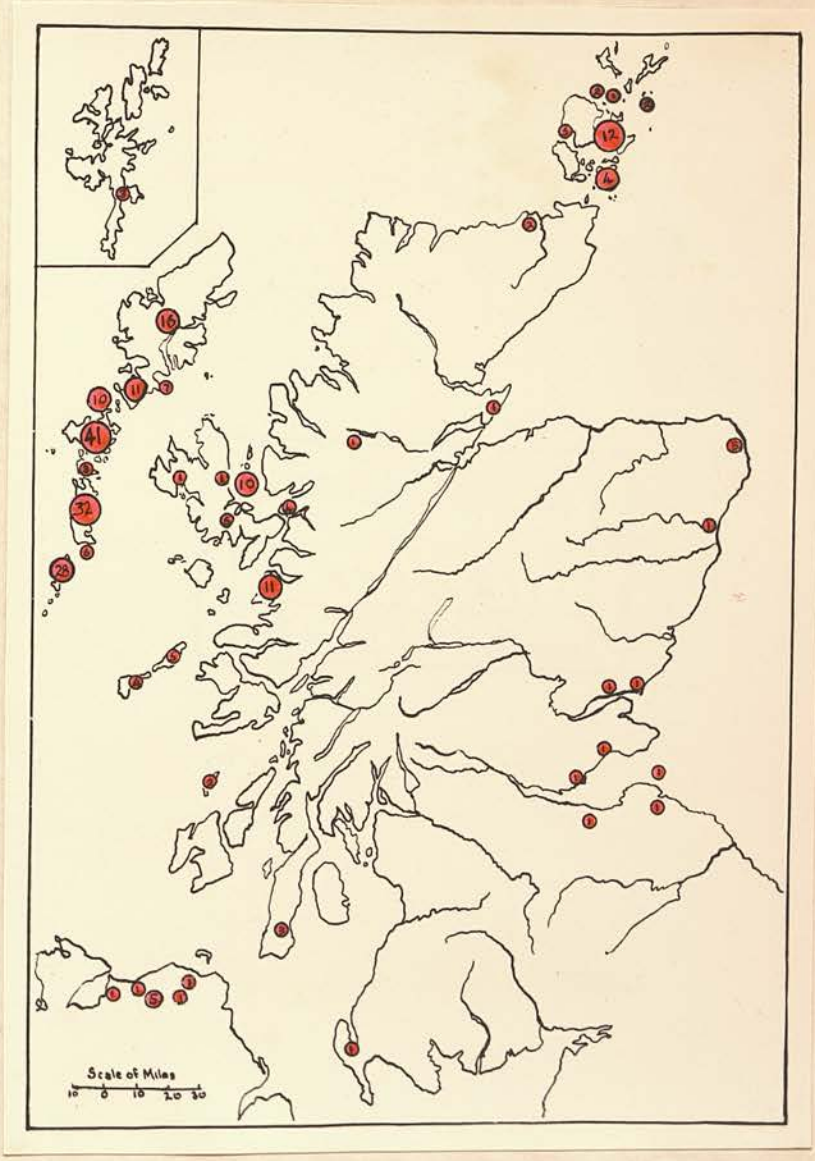
Map of Scotland showing areas to which the Congested Districts Board supplied hatching eggs or domestic fowl 1899-1900.

Blue shaded circles indicate areas to which eggs or fowls were supplied.



Map showing incidence of confirmed outbreaks of Newcastle disease in Scotland during the 1949-51 epizootic.

Red coloured circles indicate infected areas - the numbers in the circles represent the number of confirmed outbreaks in that area.



England. The most probable explanation for this circumstance being that there is relatively little importation of poultry into this country from abroad.

However on the 6th September 1949 an epizootic which was to last for 2½ years manifested itself by two outbreaks which occurred simultaneously one at Stromness, Orkney, and the other at South Ronaldsay, Orkney, there being no obvious origin for either outbreak. By the 30th September 1949 a further 38 outbreaks had been confirmed along the Scottish coastline at such diverse points as the Bass Rock (East Lothian), Cromarty (Ross and Cromarty), Lochboisdale and Eriskay (Outer Hebrides), Raasay and Tiree (Inner Hebrides), Kyle of Lochalsh and Arisaig (Inverness-shire) and in the Orkney Isles. No positive origin was established for any of these outbreaks but in view of their proximity to the sea the official attitude was that infection had occurred by the indiscriminate discharge of infected poultry offal from passing ships, or alternatively that at some infected premises, disease was being concealed and the infected poultry carcases discharged into the sea. It was presumed that wind, tide, and possibly scavenging sea gulls assisted in the further dissemination of this infective material on to the foreshores and pastures to which poultry had access.

A series of epizootiological observations and field studies were made by the author while engaged as a veterinary officer in the Lewis and Harris area of the Outer Hebrides subsequent to 8th October 1949. These are presented below.

On 15th October 1949 the author conducted an investigation into a suspected outbreak of Newcastle disease at Leverburgh, Harris (Outer Hebrides). Some 40 head of poultry were involved and they evinced all the classical symptoms and post-mortem lesions of acute Newcastle disease infection. The nervous symptoms usually described in the literature in association with less acute outbreaks were particularly marked, Opisthotonos, emprosthotonos, limb paresis, and torticollis were all observed. Some birds spun round and round in gyratory movements or flew vertically upward to a height of six to ten feet before crashing back to the ground. The classical "gasping for breath" symptom was also observed in practically all instances and there was marked diarrhoea throughout the flock. The post-mortem picture was typical with marked haemorrhages in the proventriculus, petechiae of the pleura, peritoneum and trachea, capillary haemorrhage in the subcutaneous tissue, and congestion or ulceration of the duodenum.

The owner of this flock was apparently a very

keen shot and it transpired that in the past few weeks he had confined his activities to shooting cormorants. These birds were used for domestic consumption and the offal from them was disposed of by throwing it on to the manure heap to which the domestic poultry had ready access. In the ensuing 8 weeks a further 32 outbreaks occurred in the Lewis and Harris area at widely separated points along the coast. A similar pattern of outbreaks was also occurring over a considerable part of the Scottish coastline and particularly in the North-west islands. In practically every outbreak in the Lewis and Harris area it was established that a cormorant or cormorants had been killed some 7 - 14 days previously and that the domestic fowl had either been fed the offal or had access to it. Owners of poultry stock were extremely reticent regarding disclosures of bird-shooting as many of the guns were unlicensed and also on account of the possibility of prosecutions under the Wild Birds Protection Act.

On 20th October 1952 enquiry of the local lobster fishermen regarding deaths among sea birds yielded evidence that a few dead cormorants had been observed by them floating in the sea off the small islands South-west of Harris. While this information was regarded as significant it has to

be borne in mind that there is the possibility that these birds were wounded inshore by hunters and had subsequently died and been carried away by the tide. Examination of the shores of the area for any signs of bird carcases was very disappointing, occasional skeletons or bunches of feathers from dead birds were observed but wild carrion birds and the omniverous seagulls ensured that no carcases remained identifiable for any length of time. Any extensive mortality amongst the cormorant population can be completely discounted as there was no evidence of diminution in their numbers.

Thus the evidence incriminating the cormorant as the source of Newcastle disease infection in these outbreaks appeared overwhelming. In November and December 1949 some 60 cormorants were shot by the author off the Lewis and Harris coasts. Blood samples were taken from these birds and routine field post-mortems conducted. The post-mortem examinations failed to show any macroscopic lesions whatsoever. Blood samples and a long bone, dissected from each cormorant, were dispatched to the Ministry of Agriculture's Veterinary laboratory at Weybridge for virological examination the results of which have been published, Blaxland (1951). An incidence of approximately 40% positive or doubtful H.I. titres to N.D.V. was obtained from

the cormorant sera and six virus isolations were made from the long-bones.

During this series of outbreaks an interesting observation was made regarding local spread. A disturbing feature in some of the outbreaks in remote areas had been the discovery of long-standing cases of disease. In these instances there appeared to be no deliberate concealment, failure to report disease being more a frame of mind than wilful neglect. In some cases it was procrastination, in others a desire not to cause trouble in bringing someone a long distance to investigate deaths in a few hens. These cases, however, presented a good opportunity to observe the local spread where slaughter had been delayed. The lack of local spread in such cases was remarkable, despite what appeared to be almost direct contact between healthy and diseased fowls, and innumerable human contacts. Even in cases where a number of clean flocks were mixing with infected flocks on open range for a period of weeks and were eventually slaughtered as dangerous contacts no symptoms of disease were shown. On the other hand where flocks were housed in the same building the spread of infection appeared to be extremely rapid.

To cover the background of the 1949-51 epizootic of Newcastle disease, it is necessary to

indicate the distribution and habits of the cormorant species and the areas where cormorant shooting is practised.

Despite the Biblical admonition condemning the consumption of cormorant flesh, Lev. 11. 17 "these are to be had in abomination, the little owl, and the cormorant", these birds are shot quite extensively in the Hebrides, Orkneys and to some extent in the adjacent mainland coastal areas. Two varieties of cormorant exist viz. the Greater Cormorant (Phalocrocorax carbo) and the Lesser Cormorant or Shag (Phalocrocorax aristotelis). Other sea birds which are shot for food in these areas include the Red-throated Diver (Colymbus immer) and the Great Northern Diver (Colymbus stellatus). These are of course much rarer species and were not regarded as being of significance in this investigation. In the northern part of the Island of Lewis, the young of the Gannet (Sula bassana) are shot for human consumption in considerable numbers. Wilson (1950) succeeded in isolating Newcastle disease virus from a gannet in association with an outbreak of disease among domestic poultry on the Bass Rock, East Lothian. It was interesting that no outbreaks of Newcastle disease occurred in the gannet-eating area of Lewis. Some interesting information was also obtained regarding an 'export' trade in cormorants from North-

western Scotland. It appeared that it was quite a common practice in the Mallaig-Morar area of Inverness-shire to shoot large numbers of cormorants and dispatch them to the London hotels where they were presumably fed to the unsuspecting guests as wild duck.

The cormorant is ubiquitous in all the northern seas and inland waters of any size throughout Europe, and similar forms are found in Australia, Asia, Africa and America. Birds breeding in the more northern parts tend to migrate south in the winter and there is in the British Isles a noticeable southward movement in the autumn. In the British Isles the breeding area of the Greater Cormorant is in most cases coincident with that of the Shag but in N.W. Scotland the latter bird predominates; elsewhere, as a rule, the Greater Cormorant is the more abundant species. Both species are gregarious in their nesting habits building in colonies of up to 1000 nests, packed closely on cliff ledges, especially in wave-washed caves. April is the normal month for eggs and the young fledglings usually clear the nests by August or September. Even in the winter these birds like to huddle together on the nesting ledges and make off in the mornings in small groups to the fishing zones. Cormorants can also be seen in groups in the large

inland water areas at every month of the year.

Essentially the cormorant is a fish-eater but Coward (1953) has suggested that they may have carnivorous tendencies. This is a very important observation as it gives a possible clue to the initiation of Newcastle disease infection in the cormorant colonies. In this connection it should also be noted that the cormorant's normal diet consists of small saithe, lythe, codling, eels etc. who, in their turn, will feed voraciously on flesh if given the opportunity. This presents the possibility of small fish ingesting infected poultry flesh and in turn being eaten by a cormorant who in turn could develop active Newcastle disease. The possibility of subsequent widespread dissemination of Newcastle disease in a gregarious species of this type is self-evident. It is also well-established that in parts of the Far East notably Japan and Korea the cormorant has for centuries been used as an ancillary fisherman. His capacious gullet is capable of very considerable distension and the author has shot a cormorant and subsequently recovered 21 fish from its gullet by holding the bird upside down. The Japanese fishermen maintain stocks of captive cormorants to assist them and it would seem highly probable that they would have contact with domestic poultry. The possibility

of disease developing in the cormorant species in such circumstances is obvious.

EXPERIMENTAL RESULTS:-

Experiment I To ascertain the incidence of Newcastle disease infection in cormorants on the Scottish coastline in 1952.

In 1952 32 cormorants were shot by the author in the Scottish western coastal region. Blood samples were taken from each bird and a portion of spleen and liver. Each serum sample was subjected to the H.I. test for Newcastle disease antibody according to the method of the U.S. Department of Agriculture (1946). All 32 sera were negative to the test for Newcastle disease antibody. The spleen and liver samples were pooled in small groups of 4 - 6 samples, triturated in a Ten Broeck tissue grinder, treated with penicillin and streptomycin, and inoculated into 10 day old eggs by the allantoic route. There were no deaths among the embryos after 4 days incubation and spot haemagglutination tests of harvested allantoic fluid indicated that no virus was present.

Experiment II To determine the effect of (a) feeding N.D.V. to a cormorant, (b) inoculating N.D.V. intramuscularly into a cormorant, (c) administering N.D.V. intra-nasally to a cormorant.

Six fledgling cormorants (Phalocrocorax

carbo) were caught and fed in captivity. On arrival at the laboratory serum samples were obtained from each bird and subjected to the H.I. test for Newcastle disease antibody. The test was negative in all cases. The experience gained in feeding these birds in captivity indicated that they were purely fish-eaters and they could not be induced to feed voluntarily on flesh even when subjected to long periods of starvation.

(a) Effect of Feeding: On 21/4/54 2 cormorants were forcibly fed on liver, spleen and intestines from a hen which had succumbed to infection with N.D.V. (Herts strain). Neither bird showed any indication of illness till 28/4/54 when one of the birds sickened and died that day. Post-mortem examination yielded no lesions indicative of Newcastle disease infection but N.D.V. was isolated from both spleen and liver of the dead bird. A serum sample obtained from the bird in extremis gave a negative H.I. titre for Newcastle disease. The surviving bird was also bled on 28/4/54 and a high H.I. titre was shown. This high antibody titre was maintained till 26/5/54 when it showed signs of waning and on 2/6/54 a sample of serum from this cormorant was negative to the H.I. test. Faeces samples were taken from both birds after feeding and after submitting this

material to penicillin-streptomycin treatment it was inoculated into 10 day old eggs by the allantoic route. Newcastle disease virus was isolated from samples of faeces collected on the 5th and 6th days after feeding.

(b) Effect of intra-muscular inoculation: On 23/4/54 a further 2 cormorants were inoculated intra-muscularly with 1 cc N.D.V. (Herts strain) in the form of infected allantoic fluid. These birds showed no symptoms of disease while under experiment but developed a high H.I. titre to N.D.V. 5 days after inoculation. This high antibody titre was maintained by both birds till 9/6/54 when it began to wane and on 20/6/54 serum samples taken from the birds were negative to the H.I. test. Faeces samples were taken from both these birds at varying periods and after suitable treatment inoculated into eggs. No virus isolations were effected.

(c) Effect of intra-nasal inoculation. On 10/10/52 2 cormorants were inoculated intra-nasally with 1 cc N.D.V. (Herts strain) in the form of infected allantoic fluid. There were no obvious symptoms but one bird sickened and died on 12/11/52. Post-mortem examination of this bird showed evidence of Aspergillosis but no lesions indicative of Newcastle disease. Newcastle disease virus was

isolated in eggs following inoculation of spleen and liver material from this bird. Virus was also isolated from faecal samples collected from both cormorants on 11/11/52, i.e. one month after infection and high H.I. titres to N.D.V. were demonstrated in the sera of both birds on that date.

Newcastle Disease infection of Cormorants and its relationship to the occurrence of parallel infection of domestic poultry in Scotland (1949-53).

Date	Number of Confirmed Outbreaks.	Examination of Cormorants.
1949	208	60 cormorants examined. Virus isolated in 10% Antibodies demonstrated in 40% *
1950	18	Nil
1951	10	Nil
1952	Nil	32 cormorants shot July-August 1952. No virus isolated. No antibodies demonstrable.
1953	Nil	8 cormorant bloods examined. No antibodies demonstrable.

* Virological Examinations by Blaxland (1951)

Table showing Number date and locus of outbreaks of Newcastle disease in Lewis and Harris during 1949 and the connection with the cormorant species as potential origin of disease.

No. and locus of outbreak, and date of confirmation of disease.	Origin of Outbreak
DP 5049 10/10/49 K.M., Scalpay, Harris.	These premises were adjacent and the poultry mixed in the same poultry house. Disease was well established on 10/10/49 most of the poultry being dead. Cormorant shooting was indulged in and the last cormorants had been shot 6, 14 and 18 days previously. The cormorant entrails and skins had been fed to the domestic poultry.
DP 5050 10/10/49 R.M., Scalpay, Harris.	
DP 5069 15/10/49 C.M., Leverburgh, Harris.	Cormorants had been shot 9 and 14 days prior to this outbreak and the domestic poultry had access to the entrails.
DP 5075 17/10/49 J.G., Flesherin Point, Lewis.	No admission of cormorant shooting was made by this owner.
DP 5077 17/10/49 M.M., Stockinish, Harris.	No admission of cormorant shooting was made by this owner but interrogation of neighbours indicated that a cormorant had been shot by him 10 days prior to the outbreak.
DP 5079 18/10/49 M.M., Strond, Harris.	These premises were adjacent and the owners fished and hunted together. Several cormorants were shot 7 to 10 days prior to this outbreak and their offal was fed to the domestic poultry.
DP 5080 18/10/49 A.M., Strond, Harris.	
DP 5094 22/10/49 A.C., Marvig, Lewis.	A cormorant was shot on 27/9/49 and the entrails thrown on the 'midden' which was on the edge of a field

No. and locus of outbreak, and date of confirmation of disease.	Origin of Outbreak
	of potatoes the whole being surrounded by a netting fence to prevent access by the poultry. On 8/10/49 the potatoes were lifted and the fence removed thus giving the poultry access to the cormorant entrails. Symptoms were first observed by the owner among the poultry on 20/10/49.
DP 5091 22/10/49 M.M., Northton, Harris.	A cormorant was shot by this owner 12 days prior to the outbreak. The poultry had access to the cormorant entrails.
DP 5102 25/10/49 J.A.M., Leverburgh, Harris.	No connection with DP 5069. This owner had shot a cormorant 10 days prior to the outbreak and fed the entrails to the hens.
DP 5102 25/10/49 J.M., Leverburgh, Harris.	This owner shot a cormorant 14 days prior to the outbreak and the hens had access to the entrails.
DP 5109 28/10/49 W.M., Callanish, Lewis.	This owner did not shoot cormorants but indicated that the gamekeeper, who resided on the other side of the loch at Grimersta, was very active in shooting these birds. Many of the birds shot were not retrieved and the west winds brought them across the loch to the shores of this croft. The owner stated that it was quite a common occurrence for him to find the bodies of dead cormorants washed up on his beach and he had also found them carried up on to his land by children or dogs. In these circumstances access of the domestic poultry to cormorant carcases was extremely probable.

No. and locus of outbreak, and date of confirmation of disease.	Origin of Outbreak
DP 5122 31/10/49 M.M., Garyvard, Lewis.	In this and other outbreaks in the Garyvard district the practice of cormorant shooting is so prevalent that it is difficult to establish dates of cormorant-shooting which might be of significance. Cormorant-shooting appears to be a daily occurrence and so fond are the Garyvard men of the cormorant as an article of diet that they are locally nick-named "the men of the cormorant".
DP 5137) 3/11/49 DP 5138) J.M., Scadabay, Harris. A.M., Scadabay, Harris.	These were neighbouring crofters and the owners fished and hunted together. Cormorants were shot 9, 14 and 21 days prior to the outbreak and the offal and skins had been fed to the domestic poultry in both cases.
DP 5147 9/11/49 G.M., Keose, Lewis.	There was no evidence of cormorant shooting in this area. However, Keose lies directly across Loch Erisort from Garyvard which is about 1½ miles away and the wind could readily carry dead or wounded birds across this Loch. The infected premises were on the shore and the domestic poultry ranged on the beach making a possible contact with a cormorant carcase quite probable.
DP 5148) 9/11/49 DP 5149) A.M., Garyvard, Lewis. J.M., Garyvard, Lewis.	As previously indicated the practice of cormorant-shooting in this area is so prevalent that it is difficult to indicate how many cormorants were shot by these

No. and locus of outbreak, and date of confirmation of disease.	Origin of Outbreak
	owners over the past month. Access of poultry to cormorant offal is continuous.
DP 5157 16/11/49 K.M., Kyles, Harris.	A cormorant was shot by the owner's son 11 days prior to this outbreak and the domestic poultry had access to the cormorant offal.
DP 5159 17/11/49 J.C., Lionel, Lewis.	A cormorant was shot 12 days prior to this outbreak the entrails being fed to the domestic poultry. This was one of the few families in the North end of Lewis indulging in the practice of cormorant eating.
DP 5160 18/11/49 J.M., Kyles, Harris.	This owner had been shooting cormorants frequently, the most recent birds being shot 2 and 13 days prior to this outbreak. The entrails from the shot cormorants were fed to the domestic poultry.
DP 5161 18/11/49 F.K., Marvig, Lewis.	A cormorant was shot 10 days prior to this outbreak and the domestic poultry had access to the entrails.
DP 5162 19/11/49 A.M., Swordale, Lewis.	No admission of cormorant shooting was obtained in connection with these outbreaks which occurred simultaneously on neighbouring premises. There was however a large colony of cormorants on the nearby sea cliffs. Some of these birds were shot and a high incidence of Newcastle disease antibody was demonstrated in their blood by the Haemagglutination-inhibition test. Seagulls also lived on the cliffs inhabited by the cormorants and they in turn fed regularly
DP 5163 19/11/49 W.M., Swordale, Lewis.	

No. and locus of outbreak, and date of confirmation of disease.	Origin of Outbreak
	with the domestic poultry. Direct infection by the cormorant of the domestic poultry's feeding area or mechanical transmission by the seagulls are the most probable origins.
DP 5172 19/11/49 J.M., Linshader, Lewis.	A cormorant was shot approximately 10 days prior to this outbreak and the domestic poultry had access to the entrails.
DP 5174 1/12/49 K.M., Ungishader, Lewis.	Newcastle disease had been active on these premises for some considerable time before it was discovered and in such
DP 5175 1/12/49 E.M., Ungishader, Lewis.	circumstances the origin could not be demonstrated with certainty. However in both cases the owners practised cormorant-shooting and prior to the outbreak had fed cormorant entrails to their poultry.
DP 5194 24/12/49 C.M., Gravir, Lewis.	As the author left Lewis 2 days before this outbreak occurred - no information as to origin can be presented.

DISCUSSION

Discussion.

Any individual engaged in the field control of animal virus infections cannot fail to be impressed with the dearth of positive evidence with regard to the origin of many outbreaks of disease. The origin in many of the initial outbreaks of Newcastle disease is shrouded in mystery and even the spread of disease during such outbreaks may not conform to existing ideas.

The existing conception of Newcastle disease presents the domestic fowl as the primary host with other domesticated poultry in the role of subsidiary hosts and comparatively little attention has been focussed on the possibility of wild fauna being involved in the epizootiology. Methods of spread postulated suggest that infected stock or carcase contacts are the most important factors in the spread of this disease.

Where contact between healthy and infected stock cannot be demonstrated as a likely origin recourse is frequently had to 'swill' as a possible source of infection. Examination of the literature indicates that 'swill' can be the origin of Newcastle disease infection, a typical example being the 1947 epizootic in England which was clearly traced to the feeding of poulterers' and butchers' 'swill' to poultry following the importation of infected poultry

carcases from Hungary. In many cases however it is most probable that swill is incriminated as a possible source of virus on very little evidence. In feeding any domestic animal it is usually easy to demonstrate contact with swill in some form but the question of its virus content may be doubtful. Fomites are regarded as another fruitful source of virus infection - contaminated receptacles, vehicles, feeding-stuffs, even man are blamed as mechanical carriers.

With the strains of Newcastle disease responsible for the various epizootics in Europe and Asia a short incubation period and high mortality have been the rule. This presumed a short infective period as far as the domestic fowl is concerned and one would imagine that such a disease would be self-limiting if confined to this species. It is unusual for a pathogenic organism to produce such a highly virulent disease in the main or primary host and this raises the possibility that some other species, in which infection is milder, and in which there is a lower mortality, is the primary host. Examples of this are numerous, e.g. Typhus fever has a low mortality in its primary hosts, the rat, and the rat flea but once adapted to man and the human louse it develops a very high mortality for these secondary hosts. This is taken to indicate

a more recent and less well-adapted parasitism of Rickettsia prowazeki for the human and the louse.

The 1949-51 epizootic of Newcastle disease in Scotland brought into relief most strikingly the discrepancy between the classical theories of spread of this disease and the epizootiological evidence which is presented in this thesis. Here new centres of disease developed several hundred miles away from any known active disease in poultry and as the epizootic spread connection with the sea became obvious. Sea birds appeared to be the obvious intermediary and it was the author's opinion that the conception of mechanical transmission was quite untenable.

The classical concepts of spread by direct contact or by contact with swill were readily discounted in these outbreaks. The areas affected are to a considerable extent self-supporting in so far as poultry or poultry products are concerned. As is the case on most farms it is very probable that the poultry had contact with kitchen waste and while in some areas this might be incriminated as a possible source of infection, in the islands where the disease raged with maximum intensity no imports of poultry or poultry carcases are made, and therefore the possibility of infection from such a source can be eliminated.

The evidence available points exclusively to the cormorant as the source and possibly as the reservoir of infection in the Scottish coastal outbreaks:-

1. Enquiry had established that there had been deaths in the cormorant species.

2. The geographic disposition of outbreaks indicated a sea borne infection and the density of the outbreaks corresponded with the author's personal knowledge of the incidence of cormorant shooting.

3. In almost every outbreak dealt with by the author a history of recent cormorant-shooting was obtained and the history of the outbreaks indicated that the time between exposure of the cormorant offal to the poultry and the development of Newcastle disease corresponded to the incubation period for this disease (See pages 77 - 81).

4. Infection of the cormorant species was definitely established during the epizootic (See table page 76. Among 60 cormorants shot in the area 40% had Newcastle disease antibody in their sera and 10% had virus in their long bones.

5. Examination of 32 cormorants subsequent to the epizootic showed no evidence of infection with Newcastle disease virus either in antibody tests or attempted virus isolations.

6. It is the author's submission that an outbreak of Newcastle disease occurred in 1898 with the same geographical distribution and with the same origin.

7. Experimental infection of captive cormorants showed them to be susceptible to Newcastle disease infection by the intra muscular, intra nasal and oral routes of administration and that such birds may excrete Newcastle disease virus in their faeces for a period up to 4 weeks.

8. The short duration of immunity in the cormorants under experiment as measured by antibody content of their sera indicates the possibility of repeated infection of this species analogous to repeated influenza infection in man.

The origin of disease in case DP 5094 (pages 77-81) is extremely interesting. In that case the indications are that Newcastle disease virus remained viable for 11 days in the cormorant entrails despite the exposure of this material to wind, sun, and the effect of putrefactive micro-organisms.

DPs 5162 and 5163 (page 80) are the only cases in which a mechanical transmission of virus could be postulated as origin. Of course it is just as feasible to suggest the cormorant as the direct origin in this case. The close proximity of the cormorant nesting ledges to the domestic poultry

stocks is compatible with the theory of direct transmission of infection. It has been established experimentally that the cormorant excretes Newcastle disease virus in its faeces while infected, it is also well known that cormorants frequently fly inland and that they defaecate in flight. In such circumstances contamination of the domestic poultry's feeding pasture appears a distinct possibility.

An obvious question which remains unsolved is that of the origin of infection for the cormorant. Suggestions regarding this problem have already been submitted but it must be emphasised that the low mortality and comparative mildness of the disease in the cormorant species may indicate a long-established biological adaptation of Newcastle disease virus to these birds and that the domestic fowl may in fact be only the secondary host.

While the Scottish epizootic was clearly linked to cormorant infection the possibility of other wild fauna being similarly infected cannot be overlooked. Indeed the practice of cormorant-shooting in the Scottish Islands constitutes an interesting biological indicator of the degree of cormorant infection. The evidence presented suggests that the cormorant population has only been infected twice in 50 years. It is possible that an extensive survey of other wild fauna for Newcastle

disease would be most fruitful and might disclose the true reservoir in some other host. In this connection the isolation of Newcastle disease virus from a Gannet (Wilson 1950) and from an Osprey (Zuydam 1952) is of considerable significance.

The application of the knowledge obtained in the Scottish epizootic is most interesting when related to the known world epizootiology of this disease. Examination of the world literature on geographic distribution of Newcastle Disease (pages 4 - 6) indicates a particularly high incidence of this disease in coastal areas and islands throughout the world. Unfortunately in many cases the locus of initial outbreaks are not recorded or the disease has been well established in a country before it has been recognised. In the author's opinion the locus of initial outbreaks in the following epizootics is significantly linked to the sea: Dutch East Indian Archipelago (Kraneveld 1926), The Phillipine Islands (Rodier 1928), Australia (Albiston and Gorrie 1942), East Africa (Hudson 1937), South Africa (Kaschula Canham Diesel and Coles 1946), Madagascar (Buck 1947). In all these epizootics the initial outbreaks occurred near the sea shore. Hudson (1937) commented on the occurrence of Newcastle disease in the Mombasa area reporting the disease to be enzootic in the coastal zone with extensive virulent outbreaks every

few years. Gorrie (1953) in a personal communication stated that in Victoria, Australia the initial outbreak occurred "within a stone's throw of the sea". Kaschula Canham Diesel and Coles (1946) described an outbreak near Durban, South Africa and emphasised the ease with which local spread was curtailed.

It must also be accepted that in many cases Newcastle disease has been introduced to some countries by infected fowl or carcases. Typical of this was the introduction of Newcastle disease into the Central European countries by means of importation of infected fowl from Italy. The origin of the English epizootic of 1947 has also been clearly shown to have resulted from the importation of infected poultry carcases from Central Europe (Andrews 1948). This infection has been maintained and England is not yet free from Newcastle disease. It is possible however that in the 1949-51 period some of the English coastal outbreaks were related to infection in cormorants but that the co-incident inland outbreaks marked their relationship to the sea and this resulted in all the outbreaks being shown with a common origin.

The observation of local spread in outbreaks in Lewis and Harris suggested that the incidence of mechanical transmission of infection is minimal. No evidence of lateral spread in this series of

outbreaks was obtained despite numerous human contacts between infected and healthy poultry stocks. In almost every outbreak in this area infection originated by ingestion and spread was confined to birds housed together.

SUMMARY

Summary.

1. Evidence is presented to support the contention that an extensive epizootic of Newcastle disease in fowls occurred in North west Scotland during 1897-1898. The location of the outbreaks being in the islands and coastal areas. This is the only occurrence of Newcastle disease known in Scotland prior to the 1949 epizootic.

2. The outbreaks of Newcastle disease in Scotland in 1949-51 were studied by the author while engaged in field control of this disease. The outbreaks were almost entirely confined to island and coastal areas, especially the Outer Hebrides.

3. Evidence was obtained during this epizootic suggesting that the cormorant was the source of infection of the various outbreaks in fowls.

(a) In 26 outbreaks of Newcastle disease investigated by the author a history was obtained in 23 cases that a cormorant had been shot and its entrails exposed to the poultry 8 to 12 days prior to the outbreak this being the usual incubation period of the disease. In the remaining 3 outbreaks cormorant colonies were known to live in close proximity to the feeding areas of the domestic poultry concerned and may have contaminated these

areas with their droppings.

(b) Active infection was established in the cormorant species during the epizootic. Of 60 cormorants examined during this period antibody to Newcastle disease virus was found in 40% and the virus was isolated from 10%. In 1952, one year after the epizootic 32 cormorants were examined and no antibody was demonstrable in their blood nor was virus isolated from their viscera.

(c) There was no evidence of importation of contaminated stock, nor feeding of potentially contaminated swill to the affected poultry. Lateral spread of disease from flock to flock was not observed in any outbreak.

4. In experiments with captive cormorants it was found possible to produce infection in these birds by administering Newcastle disease virus intra-nasally, intra-muscularly or orally. In all cases high antibody titres were obtained and the faeces found to contain virus up to 4 weeks.

PART II. EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

Section 1. Growth experiments in eggs and related experiments.

Experiment I. Experiment to determine the growth curves of 8 strains of Newcastle disease virus in embryonating eggs with regard to the following properties:

(a) Development of infectivity.

(b) Development of haemagglutination.

(c) Development of haemolytic activity.

(d) Killing time for the developing embryo.

Eight batches of eighty 10 day old embryonated eggs were inoculated with the eight strains of Newcastle disease virus used in these experiments. The inoculum was 0.1 ml. of a 10^{-3} dilution of pooled allantoic fluid for the appropriate strain of virus. The eggs were incubated at 37°C and every three hours three eggs were removed from each batch and held at 4°C for 2-4 hours. The allantoic fluid from each group of three eggs was harvested separately and held at 4°C for subsequent tests. From the 24th hour of incubation, the eggs were checked three-hourly by candling to ascertain the 100% death point for each strain and once this had been established no further harvests were made from that particular strain. It was essential that incubator temperatures were accurately standardised throughout this experiment as slight

variations in temperature resulted in wide variation in the growth curves of the virus strains.

(a) Infectivity of harvested fluids.

The 3 hourly harvests for each strain of virus taken from the 3rd to the 18th hour of incubation were each inoculated into 4 10 day old embryonated eggs by the allantoic route. The inoculum was 0.1 ml. of a 10^{-3} dilution. The eggs were incubated at 37° C and examined for embryo deaths at 24, 48 and 72 hours. To confirm that the deaths were due to virus activity the allantoic fluid from each embryo was harvested and subjected to the haemagglutination test. The results of this infectivity test are shown on page 100. It was noted that the strains Herts, Lasswade, Hebrides, Twiss, and Victoria caused death of some or all of the embryos with the 9th hour harvests, while the U.S.A. strains California, Massachusetts and Blacksburg caused death only with the 12th hour and subsequent harvests.

(b) Haemagglutination.

The 3 hourly allantoic harvests for each strain of virus from the 3rd hour of incubation up to the time of 100% mortality, were subjected to the standard haemagglutination test described on page 49. In all cases the erythrocytes used were from the same fowl. The results of these

tests are shown on page 101. The strains Herts, Lasswade, Hebrides and Victoria all showed haemagglutinating activity at the 15th hour and along with the Twiss strain a rapid rise in haemagglutinating titre. In the case of the U.S.A. strains the development of haemagglutination was delayed till the 18th hour and the subsequent rise in titre was more delayed, particularly with the Blacksburg strain.

(c) Haemolysis.

The allantoic fluid harvests were titrated for haemolytic activity by the method described on page 56, the haemolytic activity being expressed in terms of the difference between the two colorimetric readings. A pool of allantoic fluid from uninoculated 12 day old embryonated eggs was also included as a control. The results of this series of tests is given in the table page 102 and it will be noted that these results correspond fairly closely to these obtained with the haemagglutination titrations except that the development of the haemolytic activity appeared to be more delayed. Thus the strains Herts, Lasswade, Hebrides, Twiss and Victoria all showed haemolysis by the 21st hour and a rapid rise in haemolytic activity. The U.S.A. strain showed haemolysis between the 24th and 30th hours and the rise in

haemolytic activity was much more gradual particularly with the Blacksburg strain.

For reasons which will be shown in subsequent experiment, it was found essential to conduct these haemolytic experiments immediately after harvesting the allantoic fluids, as exposure of these fluids to storage at 4°C and even more markedly to -35°C, results in rapid increase in their haemolytic activity.

(d) Killing time for developing embryos.

All the eggs under experiment were candled 3 hourly after the 24th hour of incubation and death of the embryos established by loss of movement and by loss of definition of the blood-vessels. The time taken by the virus to cause death was found to be remarkably constant for a given strain, e.g. with the Herts strain the first deaths were noticed at the 36th hour and 100% mortality at the 42nd hour of incubation. A distinct pattern of behaviour was again observed Herts, Lasswade, Hebrides, Twiss and Victoria all showed 100% mortality of the embryos between the 42nd and 45th hours of incubation. The U.S.A. strains took considerably longer, the Massachusetts strain effecting 100% mortality at the 54th hour, the California strain at the 57th hour, and the Blacksburg strain only producing 100% mortality

after 72 hours incubation.

Experiment 2. Experiment to relate the activity of 8 Newcastle disease virus strains in ovo to their pathogenicity for adult fowl.

Three adult Rhode Island Red fowls were selected for each strain of virus and were placed in isolation. Blood samples were taken by vein puncture from each individual and subjected to the standard Haemagglutination-inhibition test as described on pages 49 - 50; all samples proving negative. The birds under test were inoculated intra-muscularly with 1 ml. of the appropriate Newcastle disease virus strain in the form of infected allantoic fluid. The results of these experiments are shown on page 104. All birds under experiment showed marked clinical signs of Newcastle disease except those inoculated with the Blacksburg strain. The birds inoculated with strains Herts, Lasswade, Hebrides, Twiss, and Victoria all succumbed to infection. With the U.S.A. strains California and Massachusetts only 1 out of the 3 inoculated birds died and with the Blacksburg strain no deaths occurred. Subsequent examination of the survivors' blood by haemagglutination-inhibition tests showed high antibody titres in the 2 survivors from California and Massachusetts infection but the Blacksburg strain produced only a minimal antibody

response, indicating a "doubtful" reaction to Newcastle disease infection.

Experiment 3. Experiment to demonstrate the effect of slight change of temperature on the rate of multiplication of N.D.V. in eggs.

The importance of maintaining the incubator at a standard temperature throughout these experiments has already been emphasised. In this experiment a batch of eggs was inoculated with the Lasswade strain of N.D.V. under conditions identical to those in experiment I except that the incubator ran at 36°C throughout. Similar tests to those described in experiment I were conducted and the results are shown in the table alongside those obtained with the same strain incubated at 37°C (Page 105). It was noted that the time of development of infectivity was prolonged from 9 to 18 hours, time of development of haemagglutination from 15 to 21 hours, time of development of haemolysis from 21 to 36 hours, and the 100% death point from 45 to 54 hours.

Experiment 4. Experiment to demonstrate the increase in haemagglutinating and haemolytic activity of virus infected allantoic fluid during storage at -35°C.

It has been pointed out in the section on

haemolysis that it is necessary to conduct the haemolytic tests immediately after harvesting the allantoic fluid as otherwise the haemolytic activity increases rapidly. In this experiment the harvested allantoic fluids for the 8 strains of virus were stored for 24 hours, 48 hours and 7 days at -35°C and tested for their haemolytic and haemagglutinating titres at these periods.

The results of these experiments are given on pages 106-9. These results showed a very marked increase in haemolytic activity as a result of storage of infected allantoic fluid at 35°C. A slight rise in haemagglutinating titre was recorded over the seven day period.

Table showing infectivity of harvested fluids.

Harvested Allantoic fluid	Time in hours					
	3	6	9	12	15	18
Herts.	0/4	0/4	4/4	4/4	4/4	4/4
Lasswade	0/4	0/4	2/4	4/4	4/4	4/4
California	0/4	0/4	0/4	4/4	4/4	4/4
Massachusetts	0/4	0/4	0/4	4/4	4/4	4/4
Blacksburg	0/4	0/4	0/4	1/4	4/4	4/4
Hebrides	0/4	0/4	3/4	4/4	4/4	4/4
Twiss	0/4	0/4	1/4	4/4	4/4	4/4
Victoria	0/4	0/4	4/4	4/4	4/4	4/4

Table showing Haemagglutination Titre of Harvested Fluids

Strains of N.D.V.	Time of incubation in hours.																						
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72
Herts	0	0	0	0	1/20	1/160	1/160	1/320	1/320	1/640	1/320	1/640	1/640	1/640*										
Lasswade	0	0	0	0	1/10	1/320	1/320	1/640	1/640	1/640	1/280	1/640	1/640	1/320	1/640*									
California	0	0	0	0	0	1/20	1/80	1/160	1/160	1/80	1/320	1/160	1/320	1/640	1/640	1/640	1/320	1/640	1/640*					
Massachusetts	0	0	0	0	0	1/20	1/40	1/80	1/640	1/320	1/640	1/160	1/640	1/1280	1/640	1/640	1/640	1/320*						
Blacksburg	0	0	0	0	0	1/5	1/5	1/10	1/20	1/10	1/10	1/10	1/20	1/80	1/160	1/160	1/160	1/320	1/320	1/640	1/320	1/640	1/640	1/3
Hebrides	0	0	0	0	1/20	1/80	1/80	1/160	1/160	1/320	1/320	1/160	1/320	1/640*										
Twiss	0	0	0	0	0	1/40	1/160	1/320	1/160	1/160	1/160	1/320	1/160	1/640	1/320*									
Victoria	0	0	0	0	1/10	1/40	1/160	1/320	1/160	1/160	1/160	1/320	1/640	1/640	1/320*									

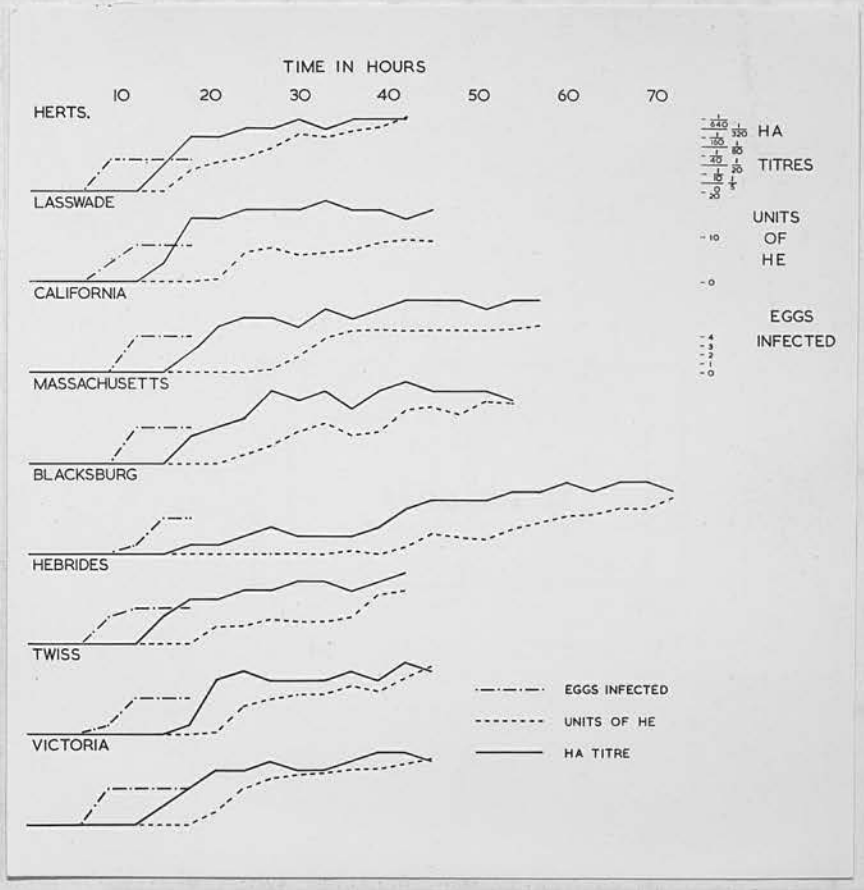
* 100% death of embryos.

Table showing Haemolytic Activity of Harvested fluids as indicated by Colorimetric Readings.

Strains of N.D.V.	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72
Herts	0	0	0	0	0	5.7	6.4	7.4	9.4	12.4	12.0	13.5	14.2	16.5*										
Lasswade	0	0	0	0	0	0	.5	6.5	7.5	6.0	6.5	7.0	8.6	9.2	9.0*									
Californ-ia	0	0	0	0	0	0	0	0	0.8	3.4	7.6	9.2	9.5	9.3	9.6	9.6	9.4	9.8	10.2*					
Massachu-setts	0	0	0	0	0	0	0	2.0	4.0	7.2	8.9	6.4	7.2	11.8	12.5	11.0	13.8	13.6*						
Blacks-burg	0	0	0	0	0	0	0	0	0	0.3	0.1	0.8	0.2	1.8	4.8	4.0	3.6	5.8	7.1	8.5	8.8	10.2	10.1	12.5*
Hebrides	0	0	0	0	0	0	3.8	4.1	5.7	5.0	5.0	6.2	11.3	12.0*										
Twiss	0	0	0	0	0	0	0.5	6.2	7.8	8.8	9.0	10.8	9.6	12.6	15.1*									
Victoria	0	0	0	0	0	0	3.0	8.0	10.2	11.0	11.6	12.2	12.4	13.5	14.5*									

* 100% death of embryos.

Graphs showing the development of infectivity, haemagglutination, haemolysis, and 100% mortality in embryonated eggs following their inoculation with 8 strains of Newcastle disease virus.



HE = Haemolysin

HA = Haemagglutinin

Table showing relative pathogenicity of 8 Newcastle disease virus for experimental fowls.

Strain of N.D.V. inoculated.	No. of birds inoculated.	No. of birds that died.	Average no. of days between inoculation and death.	Subsequent examination.
Herts.	3	3	7 days	P.M. examination showed the typical lesions of Newcastle disease.
Lasswade	3	3	5 days	P.M. examination showed the typical lesions of Newcastle disease.
California	3	1	31 days	P.M. examination of the dead birds showed lesions typical of Newcastle disease. Blood samples taken from the three birds 28 days after inoculation yielded high antibody titres on H.I. test.
Massachusetts	3	1	10 days	P.M. examination of the dead bird showed lesions typical of Newcastle disease. Blood samples taken from the surviving birds 28 days after inoculation showed high antibody titres on H.I. test.
Blacksburg	3	0	0	All 3 birds showed no symptoms of Newcastle disease. Blood samples taken 28 days after inoculation showed low antibody titres on H.I. test sufficient only to give a "doubtful" reaction.
Hebrides	3	3	5 days	P.M. examination showed the typical lesions of Newcastle disease.
Twiss	3	3	3 days	P.M. examination showed the typical lesions of Newcastle disease.
Victoria	3	3	6 days	P.M. examination showed the typical lesions of Newcastle disease.

Table showing comparative growth rates of Lasswade strain N.D.V. at different incubation temperatures.

Incubation Temperature:	37°C											36°C												
Hours of Incubation	3	6	9	12	15	18						3	6	9	12	15	18							
Infectivity for embryonating eggs.	0/4	0/4	4/4	4/4	4/4	4/4						0/4	0/4	0/4	0/4	0/4	4/4							
Hours of Incubation:	3	6	9	12	15	18	21	24	27	30		3	6	9	12	15	18	21	24	27	30			
Haemagglutinating titre.	0	0	0	0	1/10	1/320	1/320	1/640	1/640	1/640		0	0	0	0	0	0	1/5	1/320	1/160	1/160			
Hours of Incubation:	3	6	9	12	15	18	21	24	27	30	33	3	6	9	12	15	18	21	24	27	30	33	36	39
Haemolytic activity.	0	0	0	0	0	0	0.5	6.5	7.5	6.0	6.5	0	0	0	0	0	0	0	0	0	0	0.4	1.6	6.8
100% Killing time for embryos.	45 hours.											54 hours.												

Table showing rise in haemagglutination titre of allantoic harvests during storage at -35°C.

Virus Strains	Haemagglutination titre of allantoic fluid harvested between the 9th and 24th hours of incubation.						Time of storage at -35°C after harvesting.
	9 hrs.	12 hrs.	15 hrs.	18 hrs.	21 hrs.	24 hrs.	
Herts	0	1/20	1/160	1/160	1/320	1/320	
Lasswade	0	1/10	1/320	1/320	1/640	1/640	
California	0	0	1/20	1/80	1/160	1/160	
Massachusetts	0	0	1/20	1/40	1/80	1/640	0 hrs.
Blacksburg	0	0	1/5	1/5	1/10	1/20	
Hebrides	0	1/20	1/80	1/80	1/160	1/160	
Twiss	0	0	1/40	1/160	1/320	1/160	
Victoria	0	1/10	1/40	1/160	1/160	1/320	
Herts	0	1/10	1/160	1/320	1/320	1/640	
Lasswade	0	1/5	1/160	1/160	1/640	1/640	
California	0	0	1/80	1/160	1/160	1/160	
Massachusetts	0	0	1/40	1/40	1/160	1/640	24 hrs.
Blacksburg	0	0	1/5	1/10	1/10	1/40	
Hebrides	0	1/40	1/160	1/80	1/160	1/320	
Twiss	0	1/5	1/80	1/160	1/320	1/640	
Victoria	0	1/20	1/160	1/160	1/320	1/320	
Herts	0	1/40	1/640	1/320	1/640	1/1280	
Lasswade	0	1/40	1/320	1/640	1/1280	1/640	
California	0	1/1	1/40	1/80	1/320	1/640	
Massachusetts	0	0	1/40	1/80	1/160	1/1280	48 hrs.
Blacksburg	0	0	1/5	1/10	1/20	1/80	
Hebrides	0	1/80	1/320	1/160	1/320	1/640	
Twiss	0	1/20	1/80	1/320	1/640	1/320	
Victoria	0	1/40	1/160	1/320	1/320	1/1280	

Virus Strains	Haemagglutination titre of allantoic fluid harvested between the 9th and 24th hours of incubation.						Time of storage at -35°C after harvesting.
	9 hrs.	12 hrs.	15 hrs.	18 hrs.	21 hrs.	24 hrs.	
Herts	0	1/40	1/640	1/320	1/640	1/1280	
Lasswade	0	1/40	1/640	1/640	1/1280	1/1280	
California	0	1/1	1/40	1/80	1/320	1/320	
Massachusetts	0	1/1	1/80	1/80	1/320	1/640	7 days
Blacksburg	0	0	1/10	1/20	1/20	1/80	
Hebrides	0	1/80	1/160	1/320	1/320	1/320	
Twiss	0	1/20	1/80	1/320	1/1280	1/320	
Victoria	0	1/40	1/160	1/320	1/640	1/640	

Table showing rise in haemolytic activity of allantoic harvests during storage at -35°C.

Virus Strains	Incubation time of harvested eggs and haemolytic activity expressed in colorimetric units.								Time of storage at -35°C after harvesting.
	9 hrs.	12 hrs.	15 hrs.	18 hrs.	21 hrs.	24 hrs.	27 hrs.	30 hrs.	
Herts	0	0	0	5.7	6.4	7.4	9.4	12.4	
Lasswade	0	0	0	.5	6.5	7.5	6.0	6.5	
California	0	0	0	0	0	0	0.8	3.4	
Massachusetts	0	0	0	0	0	2.0	4.0	7.2	0
Blacksburg	0	0	0	0	0	0	0	0.3	
Hebrides	0	0	0	0	3.8	4.1	5.7	5.0	
Twiss	0	0	0	0	0.5	6.2	7.8	8.8	
Victoria	0	0	0	0	3.0	8.0	10.2	11.0	
Herts	0	7.8	21.4	44	50	55	62	75	
Lasswade	0	4.6	18.2	32	65	68	60	66	
California	0	0	0.3	8.6	11.4	16.5	24	30	
Massachusetts	0	0	0.8	5.2	18.5	28	36	45	
Blacksburg	0	0	0	0.8	0.3	10.5	8.4	11.2	24 hours
Hebrides	0	5.5	15.4	38	58	74	68	78	
Twiss	0	8.4	20.5	35	46	55	72	80	
Victoria	0	6.8	17.5	28.5	44	60	65	72	
Herts	0.6	27.5	64	100+	100+	100+	100+	100+	
Lasswade	0.3	0.6	60	85	100+	100+	100+	100+	
California	0	0.5	7.6	18.4	21	24	31	48	
Massachusetts	0	0.8	8.5	12.5	28	32	45	56	
Blacksburg	0	0	0.7	5.8	5.0	18.7	16.4	25	48 hours
Hebrides	0.5	22.5	56.5	100+	100+	100+	100+	100+	
Twiss	0	28.6	72	100+	100+	100+	100+	100+	
Victoria	0.8	25	64	78	100+	100+	100+	100+	

Virus Strains	Incubation time of harvested eggs and haemolytic activity expressed in colorimetric units.								Time of storage at -35°C after harvesting.
	9 hrs.	12 hrs.	15 hrs.	18 hrs.	21 hrs.	24 hrs.	27 hrs.	30 hrs.	
Herts	1.8	100+	100+	100+	100+	100+	100+	100+	
Lasswade	1.5	100+	100+	100+	100+	100+	100+	100+	
California	0.4	1.8	76	100+	100+	100+	100+	100+	
Massachusetts	0.3	2.4	88	100+	100+	100+	100+	100+	7 days
Blacksburg	0.3	0.3	8.5	46	42	100+	88	100+	
Hebrides	1.5	100+	100+	100+	100+	100+	100+	100+	
Twiss	0.7	100+	100+	100+	100+	100+	100+	100+	
Victoria	2.5	100+	100+	100+	100+	100+	100+	100+	

Section II. Virus Haemagglutination.

Experiment I. Experiment to determine the titre of haemagglutination obtained by 8 strains of N.D.V. with erythrocytes of twenty different species.

The erythrocytes from the various species were obtained by vein or cardiac puncture and were collected in citrated saline. They were washed four times before use in the tests. The haemagglutination titrations were carried out by the method described on page 49. The table page 112 shows the results obtained. It was noted that the titres obtained with avian, human, dog, Guinea pig and mouse erythrocytes gave remarkably constant results. With horse and cat erythrocytes no haemagglutination took place. The ox, sheep and pig erythrocytes showed a considerable drop in haemagglutinating titre which was particularly marked with the viruses from the U.S.A. - California, Massachusetts and Blacksburg and to a lesser extent with the Twiss strain.

Experiment 2. Experiment to determine the time of haemagglutination and elution of 8 strains of N.D.V. at different temperatures.

Pools of infected allantoic fluid were obtained for each of the 8 virus strains. In this experiment the allantoic fluids were used 7 - 10 days after harvesting. This was found necessary as fresh harvests may produce transient or imperfect

haemgglutination while older harvests appear to interfere with the stability of the erythrocytes rendering them auto-agglutinable and thus making the estimation of elution time in this test impossible. Fowl erythrocytes from the same bird were used throughout the experiment and the haemagglutination test was carried out by the method described on page 49. Haemagglutination time was taken as the earliest time at which a definite pattern of erythrocytes could be identified at the bottom of the tube and elution time as the earliest time at which the pattern disappeared and the erythrocytes sedimented into the "button" form at the bottom of the tube. The experiment was carried out at 37°C, 22°C and 4°C and the results are presented on page 113. There are obvious limitations in the technique employed in this experiment as the haemagglutinating time is closely linked to the sedimentation time of the erythrocytes. Within the limits of the technique used no significant differences in the behaviour of the eight strains of virus were observed but more rapid elution of virus was found to occur at the higher temperatures than at 4°C.

Table showing Titres of Haemagglutination produced by 8 strains of N.D.V. with erythrocytes of 20 species.

Strain of N.D.V. used	Erythrocytes																		
	Fowl	Turkey	Duck	Cormorant	Rook	Seagull	Human 'O'	Human 'A'	Human 'AB'	Human 'B'	Horse	Ox	Sheep	Pig	Dog	Cat	Guinea pig	Mouse	Rabbit	Rat
Herts	1/320	1/320	1/160	1/320	1/320	1/320	1/320	1/640	1/320	1/320	0	1/160	1/40	1/160	1/160	0	1/160	1/160		
Lasswade	1/160	1/160	1/320	1/320	1/320	1/320	1/640	1/640	1/320	1/320	0	1/320	1/40	1/160	1/160	0	1/160	1/80		
California	1/640	1/320	1/640	1/640	1/320	1/640	1/640	1/640	1/640	1/640	0	1/5	0	1/5	1/320	0	1/320	1/160		
Massachusetts	1/640	1/320	1/640	1/640	1/640	1/640	1/640	1/320	1/320	1/640	0	1/10	0	1/5	1/320	0	1/320	1/160		
Blacksburg	1/320	1/320	1/320	1/320	1/160	1/160	1/320	1/640	1/320	1/320	0	1/5	0	1/20	1/640	0	1/160	1/320		
Hebrides	1/320	1/160	1/160	1/320	1/160	1/160	1/160	1/320	1/160	1/160	0	1/80	1/40	1/80	1/320	0	1/160	1/160		
Twiss	1/160	1/80	1/80	1/160	1/160	1/160	1/80	1/160	1/160	1/160	0	1/80	1/10	0	1/160	0	1/80	1/80		
Victoria	1/160	1/160	1/160	1/160	1/160	1/320	1/160	1/320	1/160	1/160	0	1/320	1/80	1/80	1/160	0	1/160	1/160		

CELLS AUTO-AGGLUTINABLE UNDER THE CONDITIONS OF THE TEST.

Table showing Haemagglutination and Elution time of 8 strains of N.D.V. at 37°C, 22°C and 4°C.

N.D.V. strain.		Temperature at which test was conducted. 37°C	22°C	4°C
Herts.	Mean Haemagglutination Time	16 mins.	18 mins.	23 mins.
	Mean Elution Time	120 mins.	151 mins.	208 mins.
Lasswade	Mean Haemagglutination Time	15 mins.	19 mins.	22 mins.
	Mean Elution Time	110 mins.	137 mins.	183 mins.
California	Mean Haemagglutination Time	21 mins.	21 mins.	26 mins.
	Mean Elution Time	132 mins.	159 mins.	207 mins.
Massachusetts	Mean Haemagglutination Time	20 mins.	22 mins.	27 mins.
	Mean Elution Time	96 mins.	136 mins.	210 mins.
Blacksburg	Mean Haemagglutination Time	21 mins.	23 mins.	29 mins.
	Mean Elution Time	108 mins.	175 mins.	251 mins.
Hebrides	Mean Haemagglutination Time	18 mins.	22 mins.	26 mins.
	Mean Elution Time	112 mins.	145 mins.	206 mins.
Twiss	Mean Haemagglutination Time	17 mins.	21 mins.	24 mins.
	Mean Elution Time	117 mins.	118 mins.	204 mins.
Victoria	Mean Haemagglutination Time	18 mins.	19 mins.	22 mins.
	Mean Elution Time	109 mins.	114 mins.	198 mins.

Section III. Serum Haemagglutination.

The experiments are based on the work of Burnet and Anderson (1946) who demonstrated that human group 'O' erythrocytes treated with Newcastle disease virus were agglutinable to high titres either by Newcastle disease immune serum from animals or by sera from cases of infectious mononucleosis in man.

Experiment I. Experiment to determine the haemagglutination titre obtained on exposure of human group 'O' erythrocytes, modified by treatment with Newcastle disease virus strains, to sera from cases of infectious mononucleosis in man.

The method used in this experiment was that described by Evans (1950). The human group 'O' erythrocytes were treated by 8 strains of Newcastle disease virus and were then titrated against 8 sera from cases of infectious mononucleosis in man. Normal human serum was also used as a control. A table showing the results of this experiment is presented on page 117. It was noted that a similar pattern of haemagglutination titres was obtained with erythrocytes treated by the Herts, Lasswade, Hebrides, Twiss and Victoria strains. On the other hand, erythrocytes treated by the American strains, California, Massachusetts, and Blacksburg, produced

comparatively low or negative titres throughout this experiment.

Experiment II. Experiment to determine the haemagglutination titre obtained on exposure of human group 'O' erythrocytes, modified by treatment with Newcastle disease virus strains, to homologous and heterologous Newcastle disease immune fowl sera.

Following the evidence obtained in experiment I of some differences in the behaviour of erythrocytes treated by different strains of Newcastle disease virus, an attempt was made in this experiment to demonstrate strain-specificity in this reaction. Eight human group 'O' erythrocytes were modified by treatment with the eight strains of Newcastle disease virus. Each group of erythrocytes was then titrated against eight strain-specific Newcastle disease sera which had been prepared in fowls (Pages 46 - 47). The results of this experiment are presented in the table on page 115. The results were disappointing in that the haemagglutination titres obtained with the immune fowl sera were little higher than the titres obtained with normal fowl serum and there appeared to be no indication of strain-specificity about the reaction. It must be emphasised however that the immune fowl sera used in this experiment had been stored at -36°C for

for considerable periods before use (6 months to 1 year) and although the antibody content of the sera remained high under such conditions their ability to cause agglutination of treated erythrocytes may have been impaired to some extent.

Experiment III. Experiment to determine the haemagglutination titre obtained on exposure of human group 'O' erythrocytes, modified by treatment with Newcastle disease virus strains, to homologous and heterologous Newcastle disease immune rabbit sera.

This experiment was in effect a repetition of Experiment I but with rabbit immune sera substituted for the immune fowl sera. The method of preparation of the immune rabbit sera is described on page 46 and these sera were used in this experiment within 3 weeks of preparation. The titration results against the 5 strains of virus treated erythrocytes used are given in the table on page 119.
These results confirm the observation of Burnet and Anderson (1946) that human group 'O' erythrocytes treated with Newcastle disease virus were agglutinable to high titre by Newcastle disease immune serum from animals. There is no evidence in these results of any strain-specificity in this reaction.

Table showing the titre of serum haemagglutination obtained with human group 'O' erythrocytes, treated with 8 strains of N.D.V. and titrated with 8 positive Paul Bunnell and one normal human serum.

Human 'O' cells treated with 8 strains of N.D.V.	Normal Human Serum 90187	Paul Bunnell positive sera. 73334	79939	79038	80218	Baird	178828	80219
Herts.	1/10*	1/1280+	1/1280+	1/1280+	1/320	1/40	1/1280+	1/5
Lasswade	0	1/1280+	1/1280+	1/1280+	1/320	1/20	1/1280+	1/5
California	0	0	1/20	1/10	1/40	0	0	1/20
Massachusetts	0	1/5	1/40	1/40	1/160	1/10	1/5	1/40
Blacksburg	1/5	1/10	1/20	1/10	1/20	1/5	0	1/5
Hebrides	1/10	1/1280+	1/1280+	1/1280+	1/320	1/40	1/1280+	1/5
Twiss	1/5	1/1280+	1/1280+	1/1280+	1/320	1/20	1/1280+	1/10
Victoria	1/10	1/1280+	1/1280+	1/1280+	1/640	1/40	1/1280+	1/5

* Maximum serum dilution which gives agglutination of treated erythrocytes.

Table showing the titre of serum haemagglutination obtained with human group 'O' erythrocytes treated with 8 strains of N.D.V. when titrated against 8 strain-specific fowl sera and 1 normal fowl serum.

Virus treated Cells.	IMMUNE FOWL SERA								
	Herts.	Lasswade	California	Massachusetts.	Blacksburg	Hebrides	Twiss	Victoria	Fowl normal serum.
Herts	1/2560	1/5120	1/320	1/1280	1/640	1/640	1/1280	1/640	1/640
Lasswade	1/5120	1/10,240	1/320	1/1280	1/1280	1/1280	1/640	1/320	1/640
California	1/640	1/320	0	0	1/160	1/160	0	0	1/320
Massachusetts	1/1280	1/1280	1/160	1/1280	1/640	1/320	1/640	1/320	1/640
Blacksburg	1/2560	1/2560	1/80	1/640	1/640	1/320	1/640	1/640	1/640
Hebrides	1/2560	1/10,240	1/160	1/2560	1/1280	1/1280	1/640	1/640	1/640
Twiss	1/2560	1/1280	1/160	1/320	1/640	1/320	1/640	1/320	1/640
Victoria	1/2560	1/2560	1/160	1/640	1/320	1/320	1/640	1/640	1/640

Table showing the titres of serum haemagglutination obtained with human group 'O' erythrocytes, treated with 5 strains of H.D.V. when titrated with 5 strain-specific rabbit sera and 1 normal rabbit serum.

	IMMUNE RABBIT SERA					
Virus treated cells.	Herts	Lasswade	California	Massachusetts	Blacksburg	Normal rabbit Serum
Herts.	1/1280	1/5120	1/5120	1/2560	1/5120	1/320
Lasswade	1/1280	1/1280	1/2560	1/1780	1/2560	1/320
California	1/1280	1/2560	1/10,240	1/10,240	1/1280	1/640
Massachusetts	1/5120	1/10,240	1/10,240	1/10,240	1/10,240	1/320
Blacksburg	1/5120	1/10,240	1/10,240	1/10,240	1/10,240	1/160

Section IV.

HAEMAGGLUTINATION - INHIBITION.

The haemagglutination-inhibition test has been extensively used in the antigenic analysis of human influenza strains but its application to the antigenic analysis of Newcastle disease virus has been neglected. In this work an attempt has been made to utilise the techniques employed with the influenza virus to show antigenic variations in Newcastle virus strains.

Experiment I. Experiment to demonstrate the relative inhibitory effect of successive dilutions of 5 immune rabbit sera and 1 normal rabbit serum on the homologous and heterologous strains of N.D.V.

The preparation of the immune rabbit sera has already been described (page 46). Unfortunately, in the process of immunizing the rabbits, fowl erythrocytes were inoculated intra-venously along with the infected allantoic fluid and consequently these immune rabbit sera agglutinated fowl erythrocytes. It was therefore necessary to use human group 'O' erythrocytes as the indicators of virus haemagglutination throughout this test. The test method employed was that described by Salk (1944). Four minimum haemagglutinating doses (4 MHD were used throughout and the five viruses titrated against their homologous and heterologous sera.

The results are presented in the histogram, page 129 and it was observed that no definite evidence of antigenic variation of the five virus strains used was demonstrable. With the Lasswade, Massachusetts and Blacksburg strains the inhibition of virus by the homologous serum was maximal but the degree of variation in the titre of inhibition appeared in most cases to be within the bounds of experimental error.

Experiment II. Experiment to demonstrate the relative inhibitory effect of successive dilutions of immune fowl sera on the homologous and heterologous strains of N.D.V.

The preparation of the immune fowl sera has been described previously (pages 46 - 47). Erythrocytes obtained from the same fowl were used throughout this experiment. The experimental method employed was essentially that described by Salk (1944), 4 MHD of virus were used, and the 8 strains titrated against their homologous and heterologous immune fowl sera. The results are presented in the histogram, page 130. It was noted that the antibody response obtained to infection by the three U.S.A strains, California, Massachusetts and Blacksburg was considerably lower than with the remaining strains. In view of this variation in

antibody content of the immune fowl sera, examination of the columns in the histograms should be made vertically. Examination of the histogram in this way indicated that the immune sera in almost all cases exerted their maximum inhibitory effect on the homologous virus. The only exceptions to this were in the cases of Herts strain immune serum which showed a high titre against Lasswade virus and Victoria strain immune serum which showed equal inhibition with the homologous virus and with the Herts and Lasswade strains. The overall variation in inhibitory titre was slight and in all cases a considerable degree of inhibition was demonstrable with each of the heterologous strains. Repetition of this experiment on two occasions has shown these results to be reproducible within narrow limits indicating some slight variation in the antigenic structure of the virus strains concerned.

Experiment III. Experiment to demonstrate the relative inhibitory effect of 8 immune fowl sera on the haemagglutinating activity of successive dilutions of the homologous and heterologous strains of N.D.V.

The immune fowl sera used in this experiment were the same as described in experiment II except in the case of the Hebrides immune fowl serum. In this

case the original serum was accidentally contaminated and a second sample of serum was used in this experiment. The experimental method used was that described in the U.S. Department of Agriculture publication "The diagnosis of Newcastle disease" (1946) and the procedure has been outlined on pages 49 - 51. To obtain a satisfactory comparison of the 8 strains it was necessary to establish a constant dilution of serum for all strains which would give some degree of inhibition with the lower titre sera and yet would not induce complete inhibition of haemagglutination at all dilutions in the case of the higher titre sera. The most satisfactory dilution of serum in this respect was found to be 1/500 and all the sera were diluted accordingly before use. The results of this experiment are presented in the histogram, page 131. On account of the variation in antibody content in the different strain specific sera examination of the rows of columns should be made vertically. Corroboratory evidence of the strain-specificity demonstrated in experiment II was found. It was noted that in almost all cases the sera exerted their maximum inhibitory effect on the homologous virus. The only exceptions to this were in the cases of the Herts strain immune serum which inhibited haemagglutination by the Lasswade and Herts strain

of N.D.V. equally and the Hebrides strain immune serum which gave equal inhibition of the Herts and Hebrides strain of N.D.V. Repetition of this experiment on three occasions showed these results to be reproducible within narrow limits confirming that slight variation exists in the antigenic structure of the 8 strains of N.D.V. under experiment.

Experiment IV. Experiment to demonstrate the quantitative relationships of inhibition of virus haemagglutination by antiserum.

In establishing a satisfactory serum dilution for use in experiment III, H.I. tests were conducted with varying dilutions of sera. These results seemed to indicate some peculiarity in the antigen-antibody reaction in so far as haemagglutination-inhibition was concerned. When the same serum was used at different dilutions and the results converted into units of inhibition a degree of discrepancy was found. In view of this observation it was decided to perform a block haemagglutination-inhibition test with dilutions of immune fowl serum ranging from 1/100 to 1/6000 and virus dilutions ranging from 1/1 to 1/1024. The virus used in this experiment was the Victoria strain N.D.V. and the serum was homologous immune fowl serum. The

results are presented in the table, page 132 and are confirmatory of the Law of constant proportions expounded by Burnet, Beveridge, McEwin and Boake (1945) regarding the H.I. test with influenza B and the homologous immune ferret serum.

Experiment V. Experiment to demonstrate variation in antigenic structure of 8 N.D.V. strains by means of an absorption technique.

Jensen and Francis (1953) have produced an antibody-absorption technique for measuring the antigenic composition of strains of Type A influenza virus. They demonstrated that influenza virus adsorbed to erythrocytes could function efficiently in absorbing antibody from immune serum. Their method of obtaining a stable virus-erythrocyte union was by using periodate treated virus and formalinised human erythrocytes. An attempt was made in this laboratory to apply this technique to the study of Newcastle disease virus strains but was found unsatisfactory. It was observed that adsorption of N.D.V. on to the formalinised human erythrocytes was minimal. Haemagglutination tests on the virus fluids before and after adsorption indicated a drop in titre of only 1 dilution and subsequent attempts to absorb antibody from immune

sera with treated erythrocytes proved fruitless.

A method of antibody absorption for N.D.V. based on the stability of the virus-erythrocyte union at 4°C is presented.

All the reagents used were held at 4°C for 12 hours prior to the experiment. Eight test-tubes were taken and 10 c.c. of infected allantoic fluid of the appropriate strain of N.D.V. were put in each of them. To each tube was added 1 c.c. of a saturated solution of potassium meta-periodate and the mixture shaken well. 1 c.c. of washed, packed, fowl erythrocytes was added to each tube and the tubes were shaken and left at 4°C for 10 minutes. The mixture was then centrifuged, the supernatant discarded and the erythrocytes washed three times with normal saline. To each tube containing 1 c.c. washed fowl erythrocytes there was added 10 c.c. diluted strain-specific serum. This mixture was thoroughly shaken and immediately centrifuged and the supernatant of absorbed strain-specific serum collected. In this manner 8 samples of each strain-specific serum were absorbed by the 8 N.D.V. strains.

The Haemagglutination-inhibition tests were carried out by the method of the U.S. Department of Agriculture, i.e. successive dilutions of virus and standard dilution of serum. Each strain

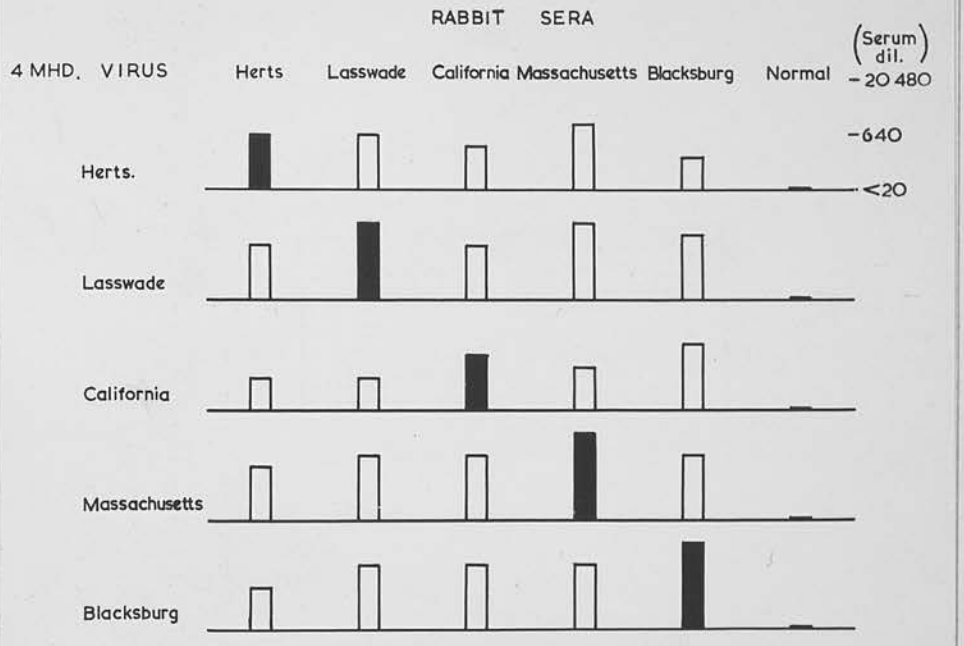
specific serum was first titrated against the absorbing virus strain. The sera were then absorbed by the method described above and tested against the same virus strain. An attempt was made to dilute the sera before absorption to a relatively standard antibody content.

The results of the H.I. tests are presented on pages 133-136. The degree of antibody absorption by a particular strain of virus was found to be maximal for the homologous serum except in the following cases. Absorption by Lasswade strain was equally effective for Herts, Lasswade and Victoria immune sera, absorption by Massachusetts strain N.D.V. was equally effective with Maesachusetts and California immune sera, absorption by the Hebrides strain was equally effective with Herts, Hebrides and Victoria immune sera and absorption with Victoria strain N.D.V. was equally effective with Herts and Victoria immune sera. The results tend to support the contention of slight variation in the antigenic structure of the 8 strains of N.D.V. under experiment.

There is a very close relationship between the strains Herts, Lasswade, Hebrides, Twiss and Victoria as their absorption pattern is very similar. The U.S.A. strains Blacksburg, California and Massachusetts are also closely

similar but there appears to be a well-defined difference in antigenic structure between the American group and the remaining strains.

Haemagglutination-inhibition tests with homologous and heterologous immune rabbit sera using a constant quantity of virus and successive dilutions of serum.

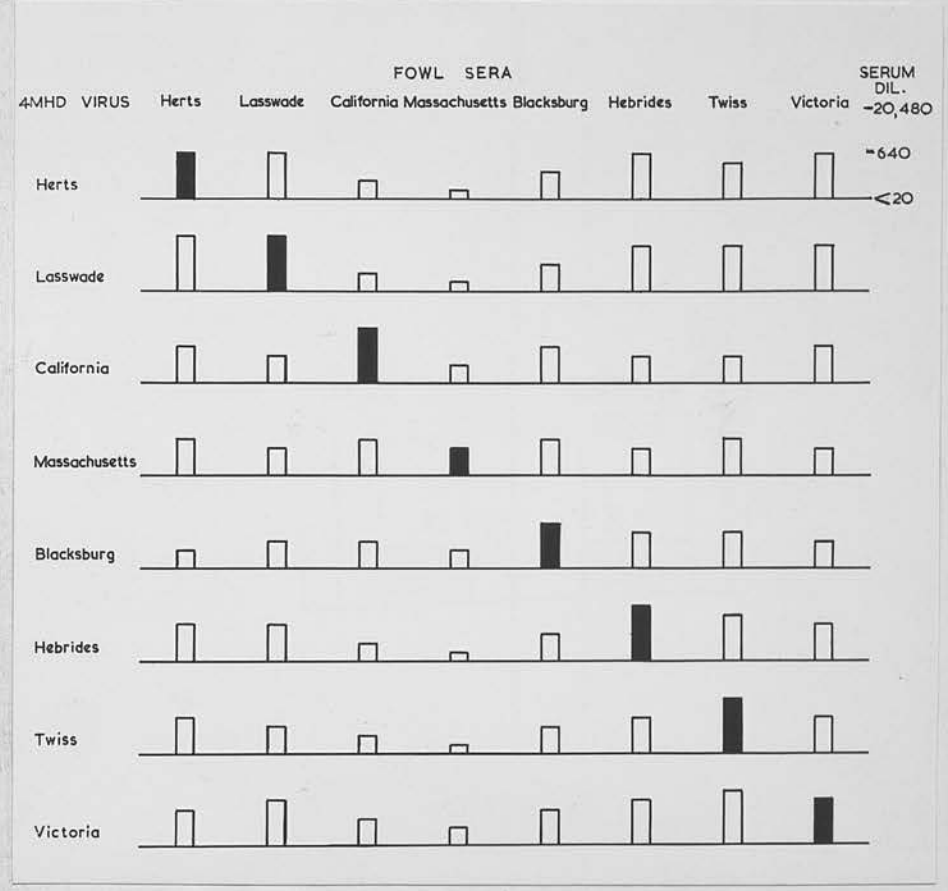


The BLACK columns represent the figures obtained with homologous immune sera.

The BLANK columns represent reactions with heterologous sera.

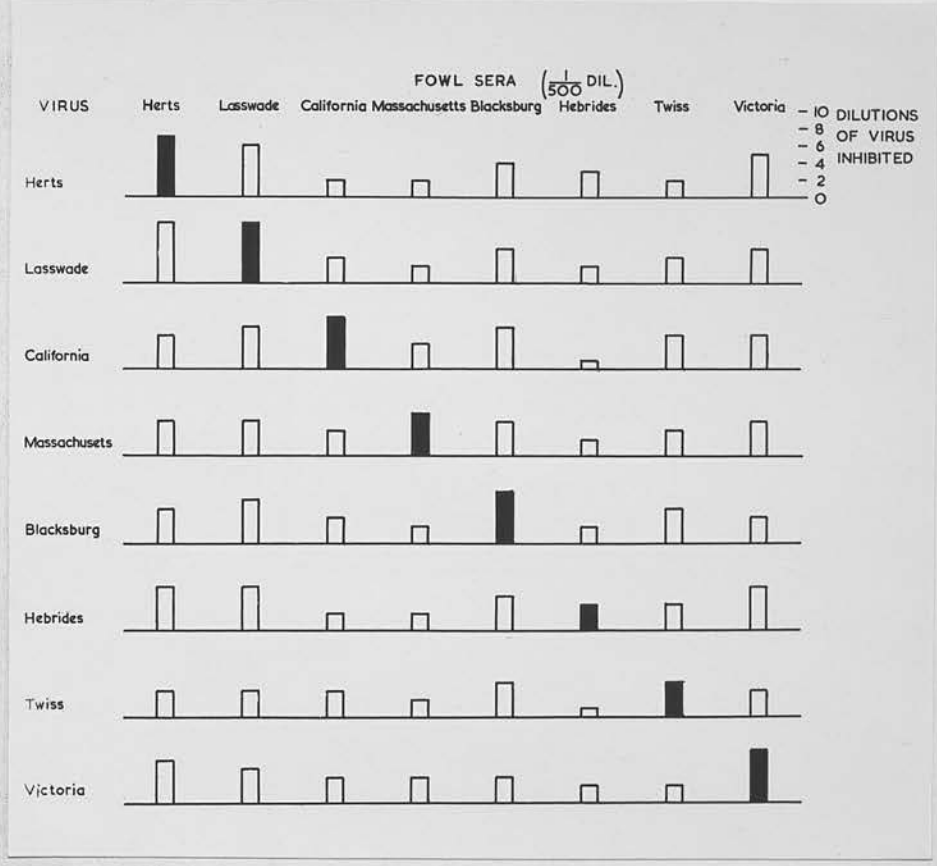
4 M.H.D. Virus represents 4 minimum haemagglutinating doses of virus.

Haemagglutination-inhibition tests with homologous and heterologous immune fowl sera using a constant quantity of virus and successive dilutions of serum.



The BLACK columns represent the degree of inhibition obtained with homologous immune sera, the BLANK columns the reaction obtained with heterologous sera. 4 M.H.D. Virus represents 4 minimum haemagglutinating doses of virus.

Haemagglutination-inhibition tests with homologous and heterologous immune fowl sera using a constant serum dilution and successive dilutions of virus.

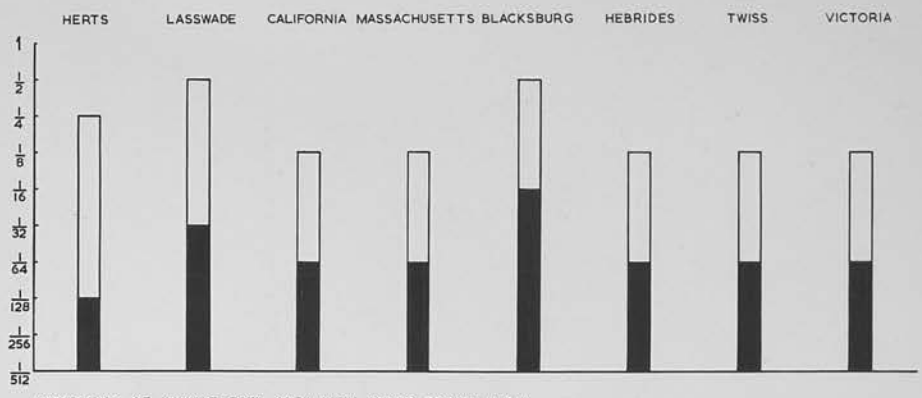


The BLACK columns represent the degree of inhibition obtained with homologous immune sera, the BLANK columns the reaction with heterologous immune sera.

Table showing result of Block Haemagglutination-Inhibition Test using successive dilutions of Victoria strain N.D.V. and successive dilutions of the homologous serum. The figures 0 - 4 indicate the degree of haemagglutination.

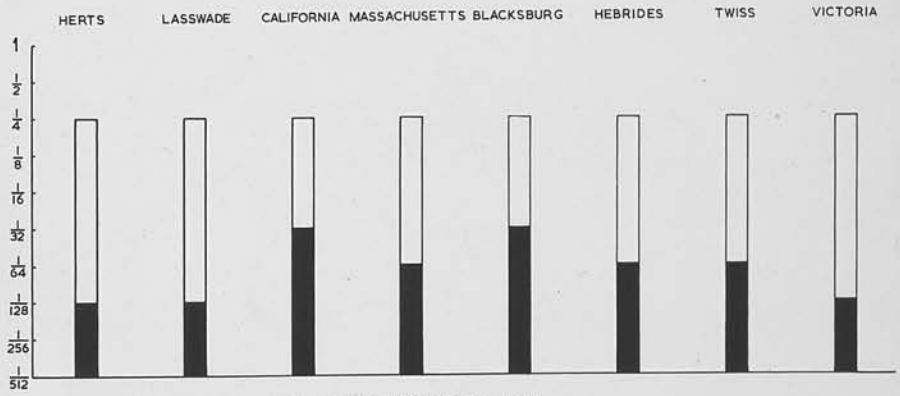
Serum dilutions	Virus dilutions 1	½	¼	1/8	1/16	1/32	1/64	1/128	1/256	1/572	1/1024	1/2028	Virus/serum Ratio at end point.
Saline (no serum)	4	4	4	4	4	4	4	4	4	2	0	0	
1/100 serum	4	0	0	0	0	0	0	0	0	0	0	0	1/100
1/200 serum	4	2	0	0	0	0	0	0	0	0	0	0	2/200
1/300 serum	4	4	0	0	0	0	0	0	0	0	0	0	2/300
1/500 serum	4	4	4	0	0	0	0	0	0	0	0	0	4/500
1/1000 serum	4	4	4	3	0	0	0	0	0	0	0	0	8/1000
1/2000 serum	4	4	4	4	4	0	0	0	0	0	0	0	16/2000
1/3000 serum	4	4	4	4	4	0	0	0	0	0	0	0	16/3000
1/4000 serum	4	4	4	4	4	4	0	0	0	0	0	0	32/4000 *
1/5000 serum	4	4	4	4	4	4	4	4	4	2	0	0	) Virus not neutral-
1/6000 serum	4	4	4	4	4	4	4	4	4	2	0	0	) :ised at these) dilutions.

* Limit of virus neutralisation.



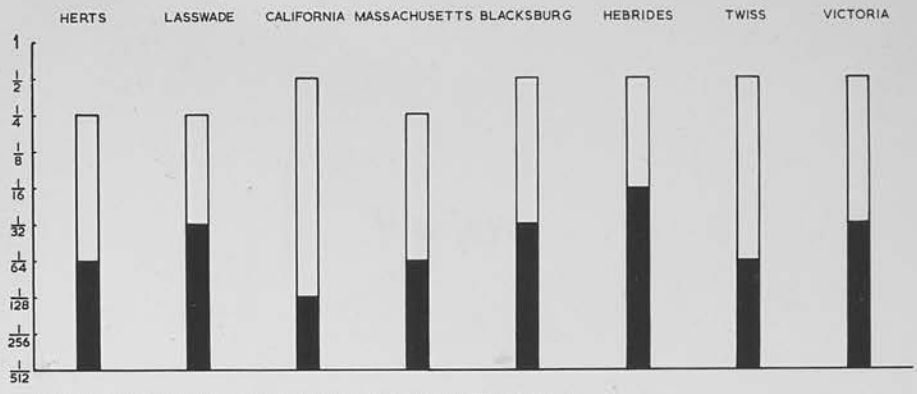
ABSORPTION OF IMMUNE FOWL SERA WITH HERTS. STRAIN N.D.V.
ALL H.I. TITRES WERE DETERMINED ON THE SAME DAY
SHADED AREAS INDICATE TITRE AFTER ABSORPTION

Histogram showing inhibition of viral haemagglutination. The columns represent the titre of inhibition of Herts strain N.D.V. by strain-specific sera before absorption. The shaded areas indicate the titre of inhibition after absorption. The figures on the left indicate the dilutions of virus.

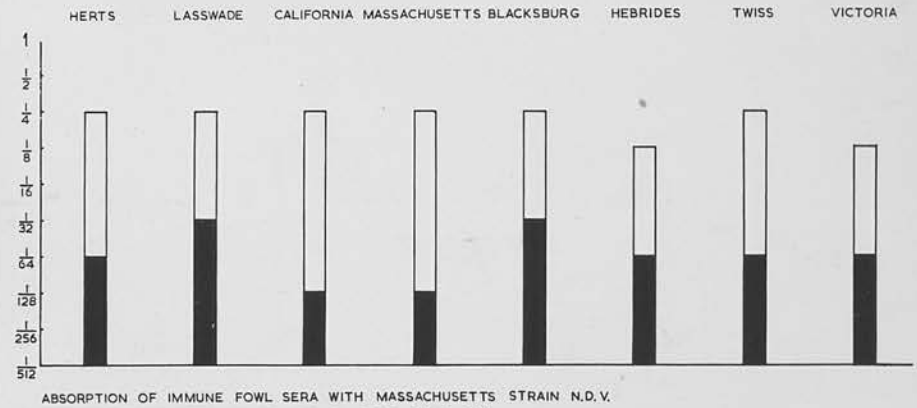


ABSORPTION OF IMMUNE FOWL SERA WITH LASSWADE STRAIN N.D.V.
ALL H.I. TITRES WERE DETERMINED ON THE SAME DAY
SHADED AREAS INDICATE TITRE AFTER ABSORPTION

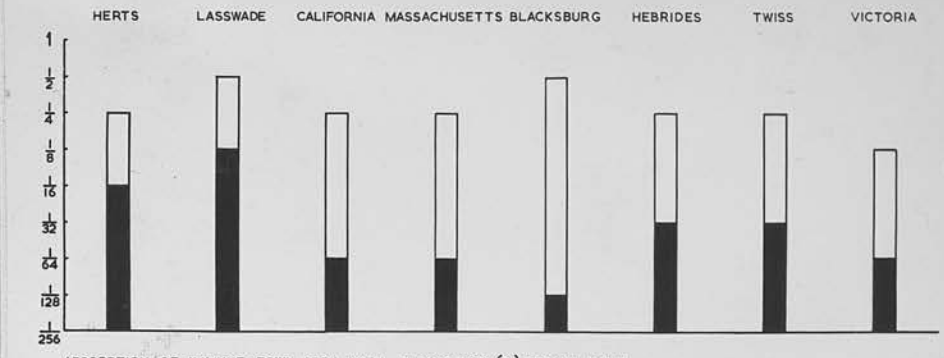
Histogram showing inhibition of virus haemagglutination. The columns represent the titre of inhibition of Lasswade strain N.D.V. by strain-specific sera before absorption. The shaded areas indicate the titre of inhibition after absorption. The figures on the left represent dilutions of virus.



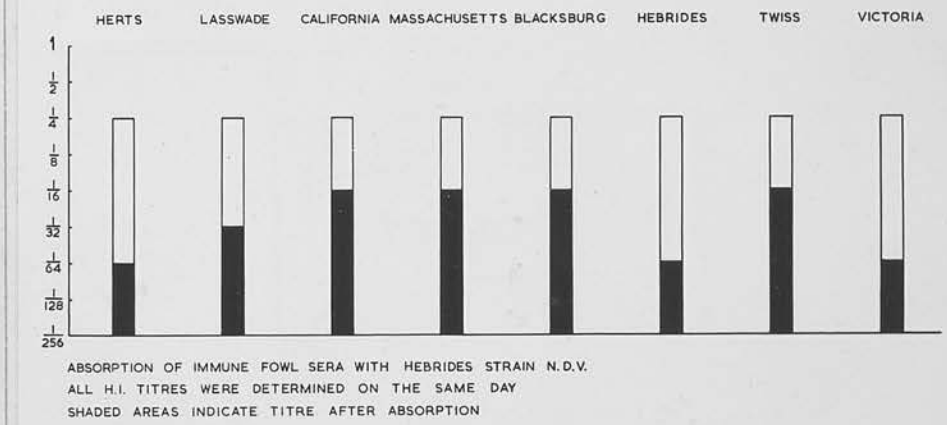
Histogram showing inhibition of virus haemagglutination. The columns represent the titre of inhibition of California strain N.D.V. by strain-specific sera before absorption. The shaded areas indicate the titre of inhibition after absorption. The figures on the left represent virus dilutions.



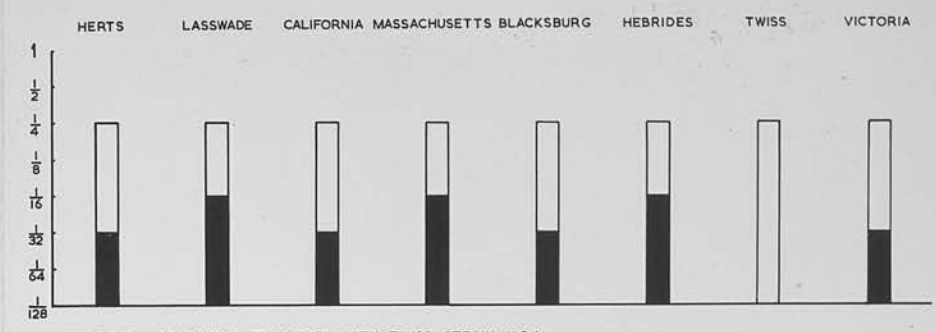
Histogram showing inhibition of virus haemagglutination. The columns represent the titre of inhibition of Massachusetts strain N.D.V. by strain-specific sera before absorption. The shaded areas indicate the titre of inhibition after absorption. The figures on the left represent virus dilutions.



Histogram showing inhibition of virus haemagglutination. The columns represent the titre of inhibition of Blacksburg strain N.D.V. by strain-specific sera before absorption. The shaded areas indicate the titre of inhibition after absorption. The figures on the left represent dilutions of virus.

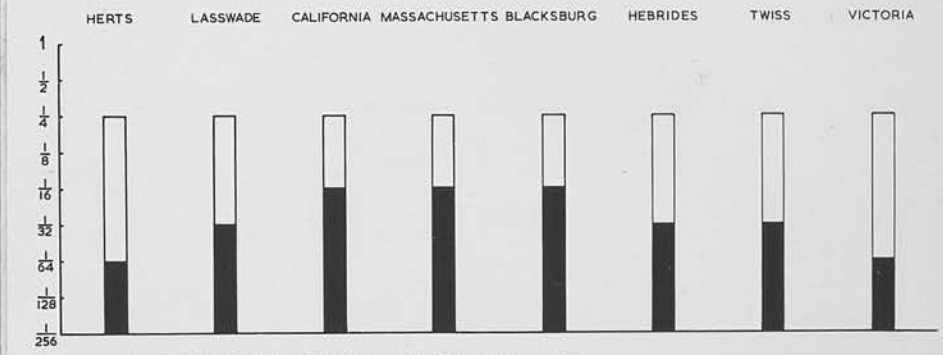


Histogram showing inhibition of virus haemagglutination. The columns represent the titre of inhibition of Hebrides strain N.D.V. by strain-specific sera before absorption. The shaded areas indicate the titre of inhibition after absorption. The figures on the left represent dilutions of virus.



ABSORPTION OF IMMUNE FOWL SERA WITH TWISS STRAIN N.D.V.
ALL H.I. TITRES WERE DETERMINED ON THE SAME DAY
SHADED AREAS INDICATE TITRE AFTER ABSORPTION

Histogram showing inhibition of virus haemagglutination. The columns represent the titre of inhibition of Twiss strain N.D.V. by strain-specific sera before absorption. The shaded areas indicate the titre after absorption. The figures on the left represent dilutions of virus.



ABSORPTION OF IMMUNE FOWL SERA WITH VICTORIA STRAIN N.D.V.
ALL H.I. TITRES WERE DETERMINED ON THE SAME DAY
SHADED AREAS INDICATE TITRE AFTER ABSORPTION

Histogram showing inhibition of virus haemagglutination. The columns represent the titre of inhibition of Victoria strain N.D.V. by strain-specific sera before absorption. The shaded areas indicate the titre after absorption. The figures on the left represent dilutions of virus.

Section V.

SERUM NEUTRALISATION.

Following the demonstration in Section IV (Haemagglutination-inhibition) of variation in the antigenic structure of the 8 N.D.V. strains under experiment, serum neutralisation tests in embryonated eggs were attempted. For reasons of economy, only 3 strains of virus and their appropriate immune fowl sera were used in this experiment.

Experiment I. To demonstrate the comparative neutralisation effect of 3 immune fowl sera on successive dilutions of their homologous and heterologous strains of N.D.V. in embryonated eggs.

The three strain-specific fowl sera used were those of the Massachusetts, California and Herts strains. The same sera were used as were described in Section IV (Experiments 2, 3 and 5) and the method of their preparation has already been indicated (Pages 46 - 47). The N.D.V. strains used were Herts, California and Massachusetts. The experimental method was that described by Cunningham (1952) and 10 day old embryonated eggs were used inoculation throughout. The test was carried out using a dilution of 1/200 of all sera and was repeated with the sera diluted 1/1000.

The results are presented on pages 139-141 and converted to histogram form on page 142.

These results indicated that the maximum neutralisation was attained by the immune sera against the homologous strain of virus in all cases, and is confirmatory evidence of variation in antigenic structure by these strains of N.D.V.

Table showing results of serum neutralisation tests in eggs with 3 strains of N.D.V. and their homologous sera. Numerator indicates number of eggs dead in 72 hours at 37°C, denominator indicates number of eggs incubated. All sera were diluted 1/200 before use.

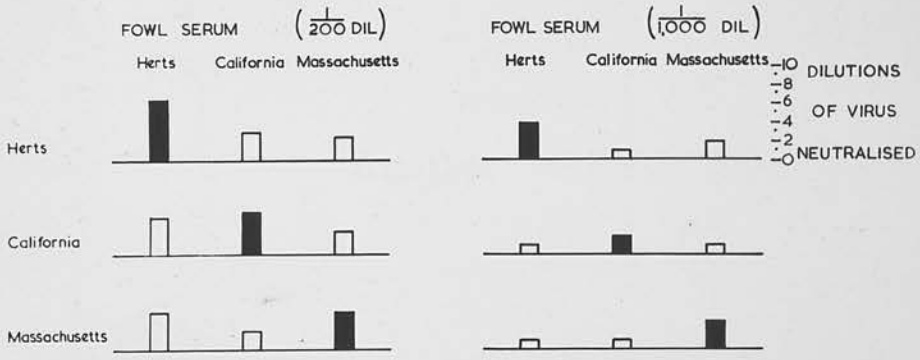
Inoculum Constituents.	Dilutions of virus inoculated								
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
.05 ml. Herts strain N.D.V. + .05 ml. saline	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	0/4
.05 ml. Herts strain N.D.V. + .05 ml. Herts strain serum.	4/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
.05 ml. Herts strain N.D.V. + .05 ml. California strain serum.	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4
.05 ml. Herts strain N.D.V. + .05 ml. Mass. strain serum.	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4
.05 ml. California strain N.D.V. + .05 ml. saline.	4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4
.05 ml. Calif. strain N.D.V. + .05 ml. Herts strain serum.	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
.05 ml. Calif. strain N.D.V. .05 ml. Calif. strain serum.	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
.05 ml. Calif. strain N.D.V. .05 ml. Mass. strain serum	4/4	4/4	4/4	4/4	2/4	0/4	0/4	0/4	0/4
.05 ml. Mass. virus + .05 ml. saline.	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4
.05 ml. Mass. strain virus + .05 ml. Herts serum	4/4	4/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4

.05/

Inoculum Constituents	Dilutions of virus inoculated								
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
0.5 ml. Mass virus .05 ml. Calif. serum.	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4
.05 ml. Mass. virus .05 ml. Mass. serum	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

Table showing results of serum neutralisation tests in eggs with 3 strains of N.D.V. and their homologous sera. Numerator indicates number of eggs dead after 72 hours incubation at 37°C, denominator indicates number of eggs inoculated. All sera were diluted 1/1000 before use.

Inoculum Constituents	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
.05 ml. Herts strain N.D.V. + .05 ml. saline	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4
.05 ml. Herts strain N.D.V. + .05 ml. immune serum.	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4
.05 ml. Herts strain N.D.V. + .05 ml. California serum	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4
.05 ml. Herts strain N.D.V. + .05 ml. Mass. strain immune serum	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4
.05 ml. California strain N.D.V. .05 ml. saline	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4
.05 ml. California strain N.D.V. .05 ml. Herts strain immune serum.	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4
.05 ml. California strain N.D.V. .05 ml. California strain immune serum.	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4
.05 ml. California strain N.D.V. .05 ml. Mass. strain immune serum	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4
.05 ml. Mass. strain N.D.V. .05 ml. saline	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4
.05 ml. Mass. strain N.D.V. .05 ml. Herts strain immune serum	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4
.05 ml. Mass. strain N.D.V. .05 ml. Calif. strain immune serum	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4
.05 ml. Mass. strain N.D.V. .05 ml. Mass. strain serum	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4



Histogram showing neutralisation effect of strain-specific immune fowl sera on the homologous and heterologous strains of Newcastle disease virus.

Section VI.

ELECTRON-MICROSCOPY.

The electron-microscope studies of Bang (1946, 1947, 1948, 1949) suggest that the Newcastle disease virus is demonstrable in two forms, viz. spherical and filamentous. He indicates that N.D.V. has a spherical form when suspended in water or .8% saline but if the ionic concentration of the saline is raised above 2% then the virus assumes a filamentous or phage-like form. Similar observations have been reported by Cunha et al (1947) and by Reagan et al (1948, 1950, 1951 I and II, 1952 I and II). It must be emphasised however that in all these publications the method used to concentrate the virus from infected allantoic fluid has been essentially one of ultra-centrifugation. The electron micrographs of N.D.V. presented by these authors in the various publications have invariably shown pleomorphic forms, chiefly phage-like in shape, but lacking in definition and frequently surrounded by detritus.

Elford et al (1948) have produced excellent electron micrographs of Newcastle disease virus concentrated by adsorption and elution of N.D.V. particles on human erythrocytes. The final suspending fluid was Ringers solution and well defined spherical virus particles were shown. Dawson and Elford (1949) evolved a new

technique of demonstrating the virus attached to a lysed fowl erythrocyte and have produced electron micrographs of lysed fowl erythrocytes with well-defined spherical virus particles attached thereto.

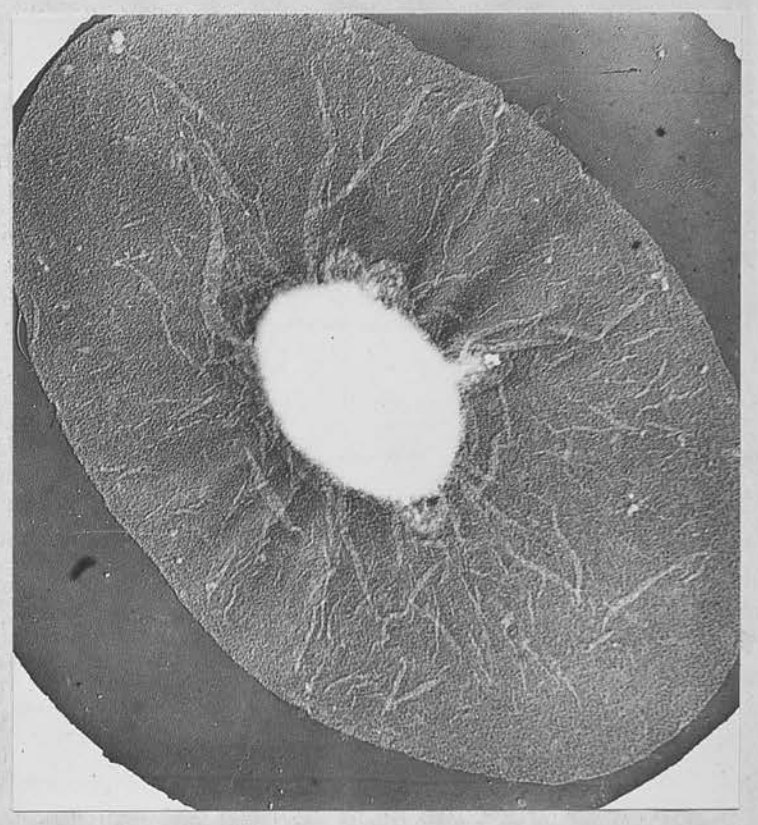
The methods used in the electron-microscope studies in this work have been based on the work of Dawson and Elford (1949) and are described on pages 57 - 59.

Three strains of Newcastle disease virus were selected for examination, viz. Hebrides, Massachusetts and Twiss. Electron micrographs were obtained of these 3 strains when adsorbed on lysed fowl erythrocytes and at varying ionic concentrations of the suspending fluid. The fixative used was .09% osmic acid. The electron micrographs are presented on pages 146-158 and all magnifications are X 10,000.

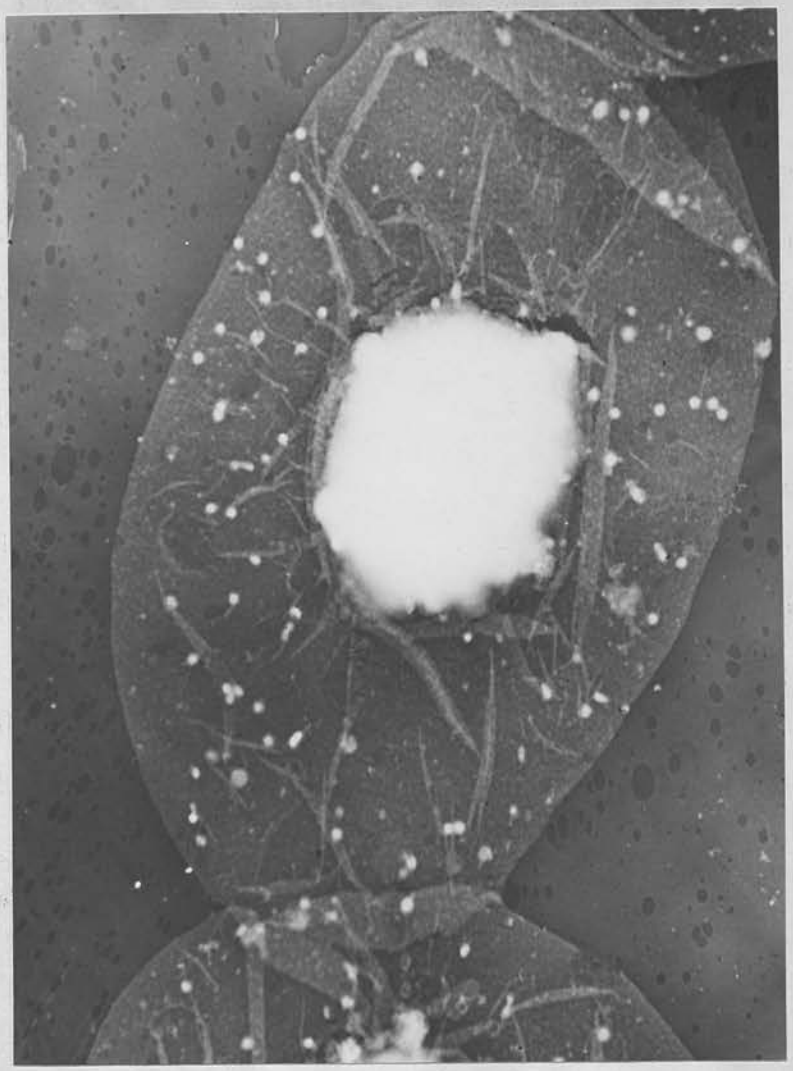
It was observed that no difference in the morphology or size of the virus particles was demonstrable between strains. With a saline concentration up to 1% the particles were relatively uniform in shape, the spherical type predominating. A small proportion of pleomorphic forms were seen, some particles being much larger than others, and a few particles appeared to have abnormal shapes. This slight degree of pleomorphism may be due to the

drying process subsequent to fixation or it may be due to the existence of dividing forms. As the ionic concentration of the suspending fluid was increased a progressive change occurred in the morphology of the virus particles which showed a tendency to elongation. At saline concentrations of over 3% the majority of virus particles assumed a cigar-shaped form but there was no phage - like or filamentous pattern as was described by the authors using ultra-centrifugation as a method of concentration. The variation in morphology of the virus particles due to change in ionic concentration of the suspending fluid probably represents a purely osmotic effect.

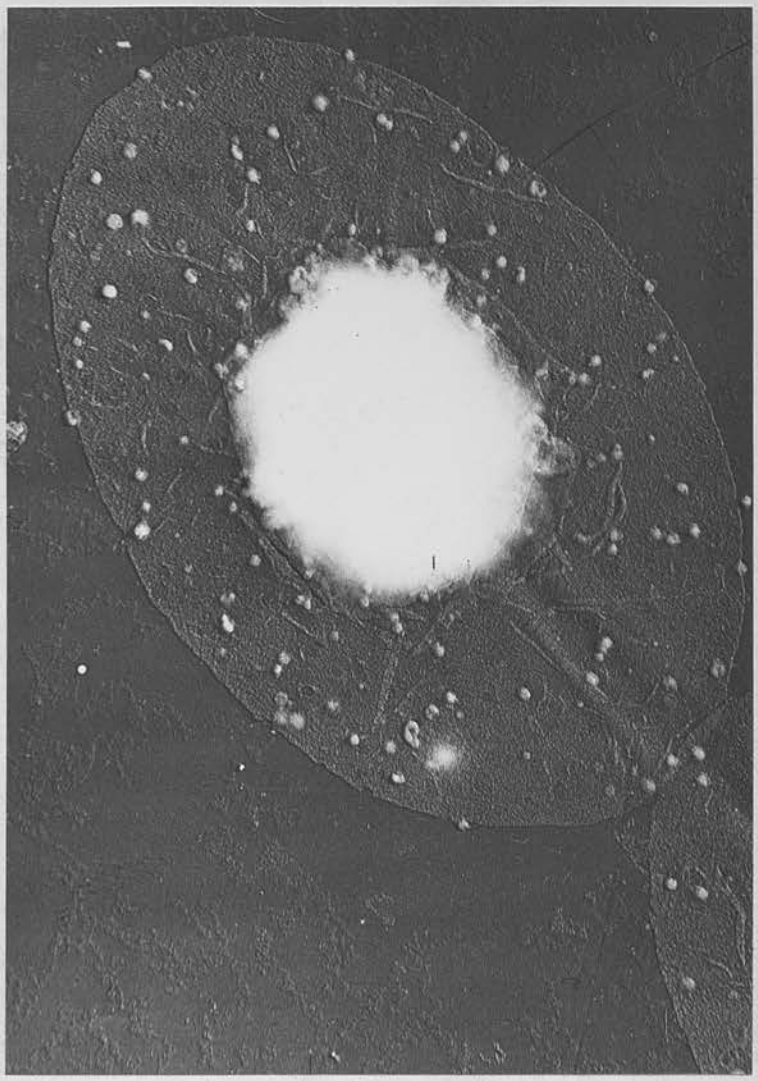
Two hundred virus particles which had been fixed at normal saline concentration of the suspending fluid were measured, and gave an arithmetic mean of 192.1 mμ the variation in particle size being approximately 140 to 240 mμ This result is in agreement with the finding of Dawson and Elford (1949).



Electron micrograph of lysed fowl erythrocyte (normal cell) **X 10,000.**

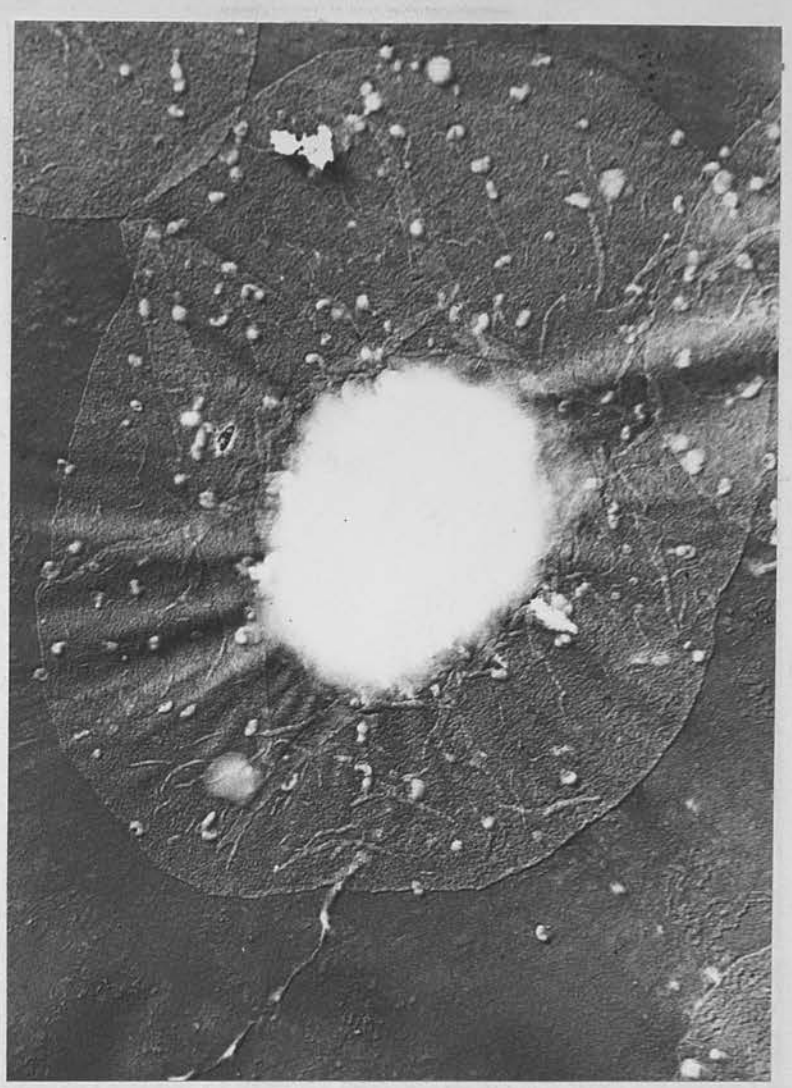


Electron micrograph of Massachusetts strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in .8% saline, unshadowed. X 10,000.



Electron micrograph of Massachusetts strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in .8% saline, and shadowed 15° Gold Palladium.

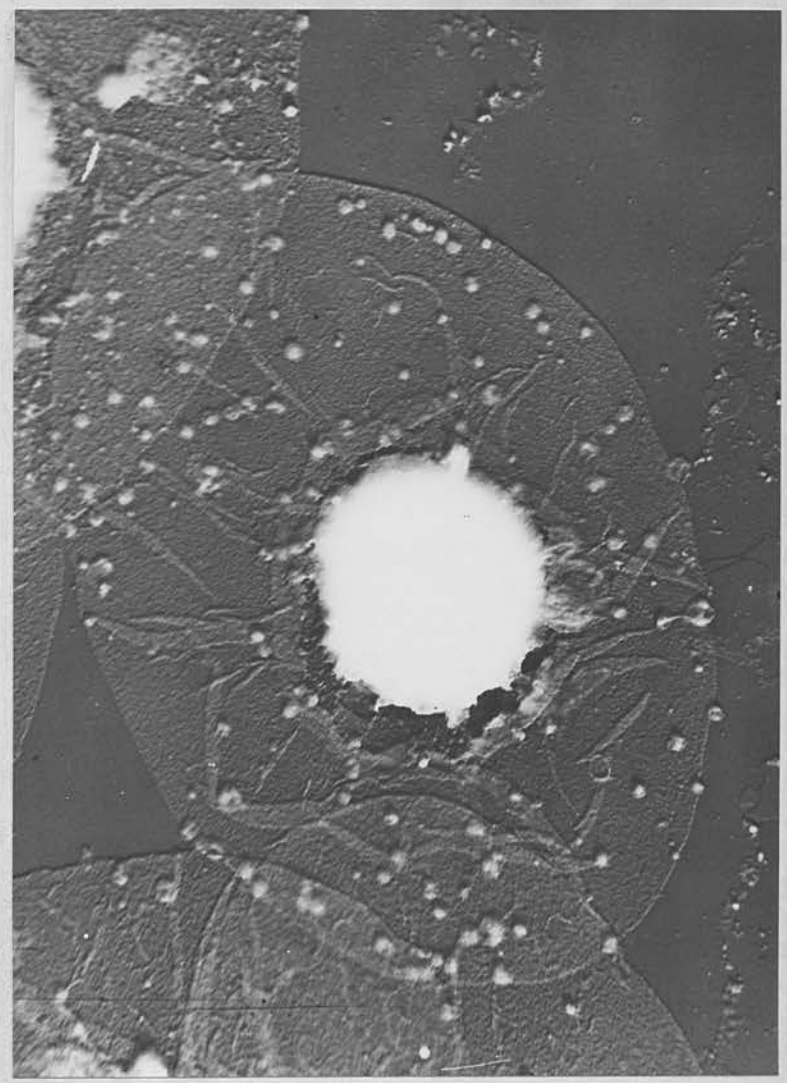
Electron micrograph of Massachusetts strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in 1% saline and shadowed 15° Gold palladium. X 10,000.



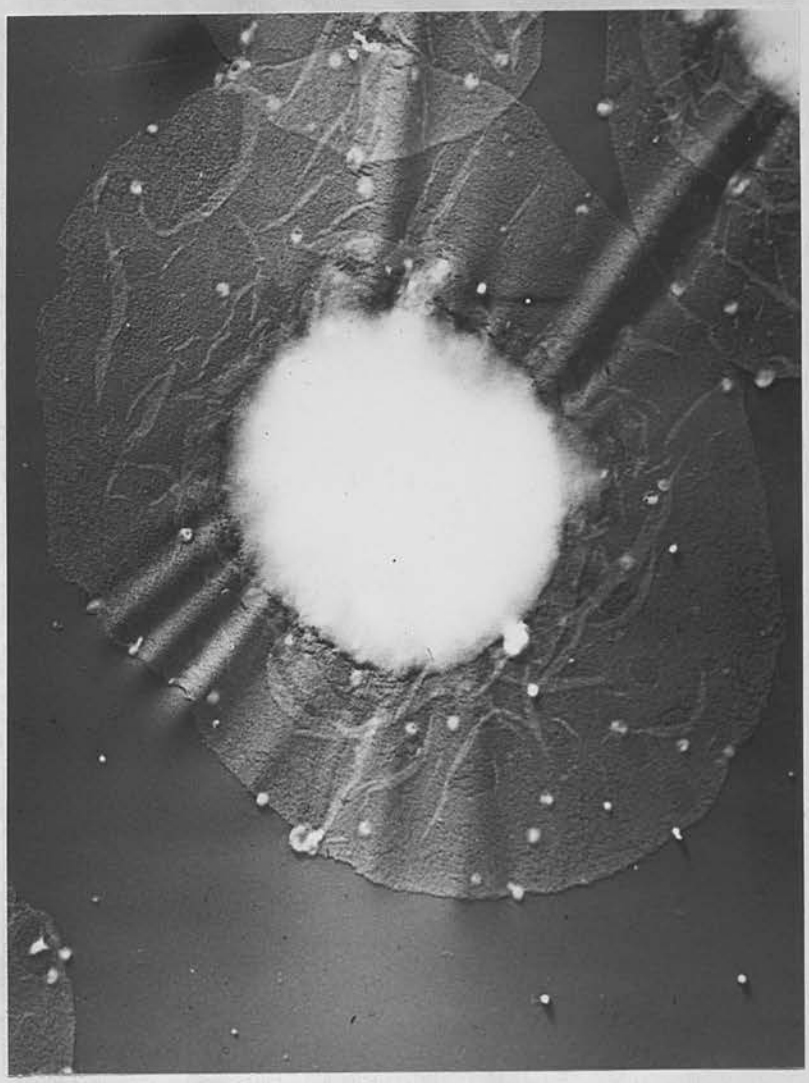
Massachusetts strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in 1.6% saline and shadowed 15° gold palladium X 10,000.

Massachusetts strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in 2% saline and shadowed 15° Gold palladium X 10,000.

Electron micrograph of Massachusetts strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in 3% saline and shadowed 15° Gold palladium X 10,000.



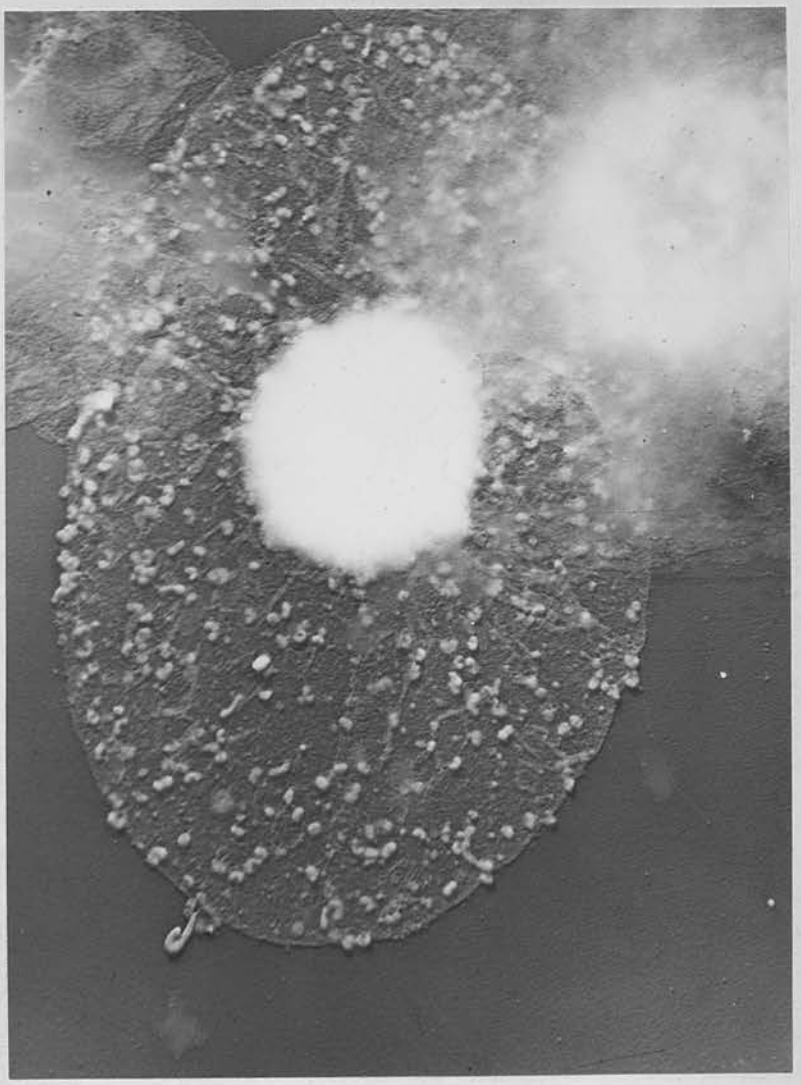
Electron micrograph of Hebrides strain N.D.V. adsorbed on a fowl erythrocyte, fixed in 1% saline and shadowed 15° Gold pall-dium X 10,000.



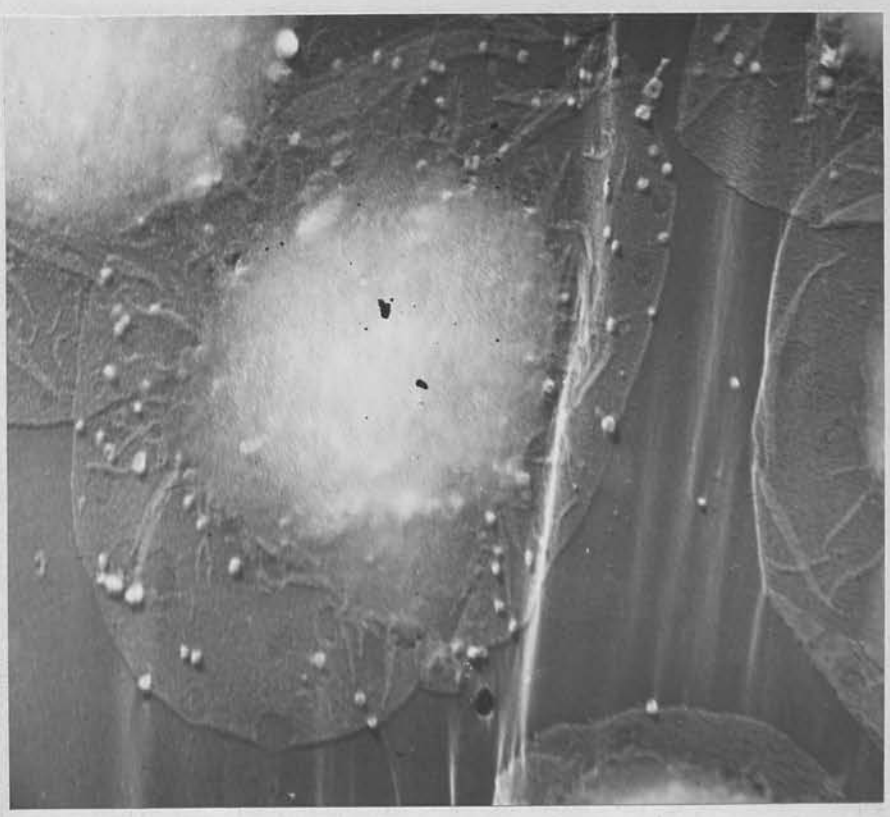
Electron micrograph of Hebrides strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in 2% saline and shadowed 15° Gold palladium X 10,000.

Electron micrograph of Hebrides strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in 3% saline and shadowed 15° Gold palladium X 10,000.

Electron micrograph of Hebrides strain N.D.V. adsorbed on a lysed fowl erythrocyte fixed in 4% saline and shadowed 15° Gold palladium X 10,000.



Electron micrograph of Hebrides strain N.D.V. adsorbed on a fowl erythrocyte fixed in 5% saline and shadowed 15° Gold palladium X10,000.



Electron micrograph of Twiss strain N.D.V. adsorbed on a lysed fowl erythrocyte, fixed in 2% saline and shadowed 15° Gold palladium. X 10,000.

DISCUSSION.

Examination of the literature relating to Newcastle disease gives a picture of 2 widely different clinical entities.

(a) The classical European or Asiatic type of infection, originally described by Kraneveld (1926) and Doyle (1927), which was characterised by hyperpyrexia, severe haemorrhagic extravasations, diarrhoea, occasionally nervous disorders, a mortality of 95 - 100% and affecting domestic fowl of all ages.

(b) The American type of infection, first observed in California in 1935 as a comparatively mild infection of domestic fowl. The name avian pneumo-encephalitis was applied to this condition by Beach (1942) and it was described as a respiratory-nervous disorder of chickens chiefly affecting birds between 2 and 10 weeks of age, but occasionally causing respiratory distress in older birds. So different were the clinical signs from those of the classical Newcastle disease that it was only in 1944 that Beach (1944) demonstrated the immunological identity of this condition with Newcastle disease. The lesions described in affected chicks were mucous exudate in the trachea and cloudiness of the membranes of the air sacs. The paramount symptoms were respiratory distress and a variety

DISCUSSION

of nervous disorders such as inco-ordination of movement, limb paresis, torticollis and opisthotonos. The average mortality was slight but on occasions acute exacerbations of disease occurred which induced a mortality of up to 50% of affected birds.

This wide variation in the clinical picture of Newcastle disease has attracted considerable attention. Burnet and Anderson (1946) have indicated the probability of two separate strains of N.D.V. and the possibility of other strains existing. Burnet (1942 and 1943) has postulated that N.D.V. and the influenza virus may have a common ancestor, basing this hypothesis on the close similarity in their haemagglutingting action on erythrocytes, and on their development in embryonated eggs. If this were the case one would anticipate as is the case of influenza virus, a considerable degree of mutation or strain differences in N.D.V. strains isolated in different parts of the world.

The great liability of viruses to variation and mutation is well-known and is strikingly exemplified in the vagaries of biological properties of the N.D.V. virus and the disease with which it is associated. The problem of strain differentiation has attracted little attention in the case of N.D. infection despite the intensive

research and voluminous literature which has been devoted to examination of the parallel infection in humans - influenza. Apparently the only investigations conducted into this aspect of Newcastle disease has been those of Jungherr et al (1946) and Brandly et al (1946).

Jungherr et al (1946) investigated the comparative pathology of infections produced by 9 strains of N.D.V. (5 of American origin and 4 of European origin), and their findings indicate that the European strains were primarily viscerotropic while the American strains were pneumotropic or neurotropic depending on an intra tracheal or peritoneal avenue of infection. In the embryonated egg the European strains caused marked chorio-allantoic lesions particularly in older embryos, and capillary haemorrhage in the skeletal muscles while the American strains tended to produce such haemorrhages in the central nervous system. Brandly et al (1946) carried out cross neutralisation tests in eggs with several pneumo-encephalitis strains from California and an English 'E' strain. They found that the maximum virus inactivation was usually obtained with the homologous serum. They also indicate that some degree of strain-specificity was demonstrable by comparative haemagglutination-

inhibition tests.

In view of the world-wide distribution of Newcastle disease and of the extensive use of live avirulent strains of virus as immunizing agents, the importance of thorough examination of Newcastle disease virus strains can hardly be over-emphasized. There have been disturbing reports of the inefficacy of some avirulent strains as immunizing agents. For example Blanco (1949) described the advent and spread of N.D. infection throughout Spain. Vaccines of American origin failed to protect fowls against the indigenous virus although vaccines prepared from local strains by chick embryo passage apparently proved effective.

The major direction in this study has been to compare 8 strains of N.D.V. isolated in widely separated parts of the world with the view of demonstrating differences in their antigenic structure or their biological behaviour.

Considerable differences were demonstrated between the 8 strains of N.D.V. as regards their growth and development in embryonated eggs. In this section attention was focussed mainly on the development time for the various virus strains having regard to their infective, haemagglutinating, haemolytic and lethal properties. The experimental findings summarised in the graphs

page 103 placed the 8 virus strains in 3 groups.

1. The highly virulent strains. This group included Herts, Lasswade, Hebrides, Twiss and Victoria strains and all of them presented a very similar picture as regards their activity and development in embryonated eggs. Any slight variation in their activity can be accepted as being within the bounds of experimental error.

2. Strains of medium virulence. This group included both strains isolated in the U.S.A. viz. California and Massachusetts and again a very similar pattern of behaviour was observed in their development in the embryonated egg. A marked time difference exists in the development of all the properties of this group of strains as compared with groups (1) and (3).

3. Strains of low virulence. This group is exemplified by the Blacksburg strain and was characterised by very slow development of all virus properties. In fact the lethal time for this virus was almost double that found under identical experimental conditions with group (1). Similarly the time of development of infective, haemolytic and haemagglutinating properties was much longer with this virus than with any of the other strains.

The relationship between pathogenicity in the adult fowl and that in the developing embryo has also been investigated and there is a definite

correlation between the two. A slight difference in pathogenicity was demonstrable between the individual members of group (1) as far as adult fowl were concerned but it must be admitted that standard experimental conditions are much more difficult to maintain in this type of experiment.

Observations made in the course of the egg inoculation experiments throw doubt on the controversial findings of Nadel and Eisenstark (1955) that in the development of the California strain (11914) of N.D.V., the production of haemagglutinin preceded the development of infectivity in harvested allantoic fluids from inoculated embryonated eggs. This apparently paradoxical observation is at variance with the egg-inoculation experiments detailed in this work and also with the findings of Gordon et al (1952). Throughout this investigation it was found that the greatest dilution of infected allantoic fluid which would induce haemagglutination was in the region of 1/2000. On the other hand .05 ml. of 10^{-8} infected allantoic fluid will prove infective for the developing embryo. This indicates that a fraction of the quantity of infected allantoic fluid necessary to produce haemagglutination will infect an embryonated egg, and these figures approximate to those given by Burnet (1942). It has also been demonstrated in

this investigation that the haemagglutinating and haemolytic properties of N.D.V. in infected allantoic fluid increase as a result of storage at -35°C. This observation confirms the findings of Anderson (1947) regarding increase of haemagglutinating titre of infected allantoic fluids following storage. Anderson postulated that this rise in titre was due to the break-up of aggregations of virus particles, an observation which is in accord with the evidence presented in this work. The same reason is advanced to explain the increase in haemolysis indicated in the experiments on stored virus. It may well be that this phenomenon is the cause of the discrepancy in the results of Nadel and Eisenstark.

The investigations of virus haemagglutination has shown a pattern of activity by the different strains which is not without significance. It was noted that all the strains gave high haemagglutination titres with all the avian and human erythrocytes. It was also observed that the N.D.V. strains Herts, Lasswade, Hebrides, Twiss and Victoria gave on the whole a considerably higher titre than did the U.S.A. strains with the remaining mammalian erythrocytes.

Thus the different strains of N.D.V. distinguished by different degrees of pathogenicity

in the field did show differences experimentally:

1. In their growth and activity in eggs.
2. In their pathogenicity to experimentally inoculated fowls.
3. In their haemagglutinating activities.

Having regard to the O - D variation in haemagglutinating property of the influenza virus, described by Burnet and Bull (1943) it seems possible that this variation in haemagglutinating titre of N.D.V. strains with mammalian erythrocytes may indicate a widening of the host range of some of the virus strains to include these species. The wider prevalence of Newcastle disease infection in poultry throughout the world has increased the opportunity for more abundant and extensive exposure of other species mammalian as well as avian. The pathogenicity of N.D.V. for the human species is well established (Burnet 1943, Anderson, 1946, Shimkin, 1946 and Yatom, 1946). Possibly indicative of the predictable future behaviour of the virus to species at present considered resistant is the adaptation of the California strain of N.D.V. to serial passage in, and fatal infection of, the hamster by Regan et al (1947 and 1948). A further report by Wenner and Lash (1949) of inoculation infection in monkeys indicates that N.D.V. has

adequate potentiality for expanding its host range in the future.

The observations on serum haemagglutination of N.D.V.-treated erythrocytes is also interesting in this respect. The high titres of haemagglutination found with positive Paul Bunnell sera and N.D.V. treated human group 'O' erythrocytes can hardly be presented as a true antigen-antibody reaction. Nevertheless, as has been suggested by Burnet and Anderson (1946), it is not inconceivable that a strain of N.D.V. could be responsible for a human infection (glandular fever) spread by the respiratory route.

The observations on serum haemagglutination are also interesting in regard to strain differences. The erythrocytes treated with Herts, Lasswade, Twiss, Hebrides, and Victoria strains of N.D.V. all gave high titres with the positive Paul Bunnell sera used, while the erythrocytes treated with the U.S.A. strains gave comparatively low titres. The lack of strain-specificity in this reaction was amply demonstrated in the experiment with strain-specific immune rabbit and fowl sera. It appears that the knowledge of the exact nature of this reaction is incomplete and attempts to draw exact conclusions from these results dangerous.

The observations on serum neutralisation and haemagglutination-inhibition and inhibition

absorption tests call for little discussion. Throughout these experiments ample evidence of minor difference in the antigenic structure of the 8 virus strains is offered. The experiments indicate also that a high degree of cross-immunity exists between the 8 strains of N.D.V. under experiment and the results accord with the observations of Brandly et al (1946). Examination of the results indicates an especially close relationship between the three U.S.A. strains as compared with the strains isolated in other parts of the world and this may be due to a different source of infection as regards the U.S.A. epizootic. The failure to utilise the technique of Jensen and Francis (1953) in the antibody absorption studies was due to inability to get a stable virus-erythrocyte union between the periodate-treated N.D.V. particles and formalinised human erythrocytes. A method based on virus-erythrocyte union at 4°C was evolved and has proved satisfactory.

The study of the various strains of Newcastle disease virus in the electron microscope clarifies our knowledge of the morphology of this virus. Most of the existing electron-microscope studies on N.D.V. have used ultra-centrifugation as the method of concentrating and purifying the virus for examination. Bang (1946) has demonstrated filamentous forms using

this method and subsequently Bang (1947) demonstrated variation in virus conformation from the spherical to the filamentous depending on the ionic concentration of the suspending fluid. Reagan et al (1952) using a similar technique to prepare the virus for examination claimed that the variation in shape of the virus was associated with age. They suggest that early in growth the virus particle is spherical and that later filamentous prolongations develop. Reagan et al (1950, 1951 and 1953) make further claims that passage of the virus through such diverse hosts as the mouse, cave-bat and green turtle results in modification of its tail structure.

It is fairly obvious that ultra-centrifugation used asa method of concentrating virus is liable t o distort the morphology by physical stress and thus lead to false conclusions as their is quite a possibility of producing aberrant forms or even artefacts by this technique. Accordingly the method of concentrating virus used in this study was that of Dawson and Elford (1949) the virus being adsorbed on lysed fowl erythrocytes and fixed with osmic acid. This method ensures a minimum amount of physical damage to the virus and a much more uniform pattern is obtained as regards morphology. The electron microscope studies indicatedno

appreciable differences between the virus strains examined nor could any variation in virus morphology associated with age of the harvest be demonstrated. Some degree of variation in morphology of the virus was evident when fixing was carried out at different ionic concentrations of the suspending medium. When the concentration of saline in the suspending medium was raised above 2% a distinct tendency to elongation on the part of the virus particles was observed. This observation is in part confirmatory of the observations of Bang (1947) using the ultra centrifugation technique. Frankly filamentous or sperm-shaped forms were not generally observed the most typical shape produced being sausage-like. There was however a considerable degree of pleomorphism at the higher saline concentrations. It will be appreciated that differentiation of strains on the basis of tail modifications in these circumstances was difficult. The electron microscope studies undertaken in this work suggest strongly that the N.D.V. particle is normally spherical and that alterations in the shape of the particle are purely the result of physical forces, the probable mechanism being that the virus particle has a definite limiting cell membrane and exposure to high ionic concentrations results in fluid being

absorbed from the particle by the simple process of osmosis.

SUMMARY

SUMMARY

Eight strains of Newcastle disease virus isolated during epizootics in different countries and of different degrees of virulence were examined for demonstrable differences by laboratory methods. These included 5 strains producing the classical European or Asiatic syndrome characterised by high mortality viz. Herts (U.K.), Lasswade (U.K.), Hebrides (U.K.), Twiss (Canada) and Victoria (Australia). The less virulent American type of virus was represented by two strains of medium virulence, California and Massachusetts, and also by the avirulent Blacksburg strain which is used for vaccination purposes in the U.S.A.

These strains were examined as regards

I. Rate of growth in the allantoic cavity of embryonated eggs with regard to:

(a) Infectivity.

The first property to develop in the allantoic fluid of inoculated eggs was that of infectivity. Following 9 hours incubation the strains Herts, Lasswade, Hebrides, Twiss, and Victoria showed some degree of infectivity. The three U.S.A. strains did not show this property till the 12th hour of incubation. There thus appears to be no eclipse phase or soluble form in the case of N.D.V. development because infectivity precedes the development of haemagglutination and this constitutes

a major difference between Newcastle disease virus and the influenza virus.

(b) Haemagglutinin production.

Haemagglutinin was demonstrable in the allantoic fluid of infected eggs at the 15th hour in the case of the strains Herts, Lasswade, Hebrides and Victoria. Haemagglutinin was demonstrable with the remaining strains at the 18th hour of incubation.

(c) Haemolysin production.

Haemolysin was demonstrable in the allantoic fluid of infected eggs at the 21st hour in the case of strains Herts, Lasswade, Twiss, Hebrides, and Victoria, at the 24th hour with Massachusetts, at the 27th hour with the California strain and at the 30th hour with the Blacksburg strain.

(d) Lethal properties for the inoculated egg.

The highly virulent strains all produced death of the developing embryos by the 45th hour of incubation. Massachusetts produced death at 54 hours, California at 57 hours and the Blacksburg strain after 72 hours.

Experimental inoculation of adult fowls with the eight strains of N.D.V. showed a close correlation between the pathogenicity of the individual strains for adult fowl and their activity in the embryonated egg.

II. Haemagglutinating properties of the virus.

The haemagglutinating activity of the 8 virus strains against 20 different types of avian and mammalian erythrocytes showed on the whole a similar pattern. A high titre was obtained with all the strains against avian, human, guinea-pig and mouse erythrocytes. With ox, sheep, and pig erythrocytes a very low or negative titre was shown by the U.S.A. strains and a substantial titre with the other strains. There was no agglutination of horse or cat erythrocytes by any of the strains. Within the limits of the experimental method employed no significant differences in haemagglutinating or elution time for the 8 strains were established. No O - D variation analogous to that experienced with influenza virus was demonstrable.

III. Action in altering agglutinability of human erythrocytes by certain sera.

Agglutination of N.D.V.-treated erythrocytes by positive Paul Bunnell sera accentuated the difference between the U.S.A. strains and those isolated elsewhere in the world. In practically every case, low or negative titres were obtained when erythrocytes treated with the U.S.A. strains were used, while the use of erythrocytes treated by any of the other 5 strains resulted in high titres being obtained in most cases. Repetition of this experiment

using N.D.V. immune animal sera in place of Paul Bunnell sera showed no such strain-specificity.

IV. Virus susceptibility to neutralisation by antisera as demonstrated by

(a) Haemagglutination-inhibition and H.I. absorption tests.

(b) Virus neutralisation tests in ovo.

Haemagglutination-inhibition tests using N.D.V. immune fowl sera indicated that such sera exerted a greater inhibitory effect against the homologous strains of virus than against the heterologous strains, thus indicating differences in the antigenic structure of the virus strains. However, as there was a marked degree of cross-neutralisation it appears that these differences were slight. H.I. antibody absorption tests confirmed the view that there were slight differences in antigenic structure between the strains. In their behaviour in this series of tests the U.S.A. strains again showed a closer resemblance to one another than to the remaining strains. The Herts and Lasswade strains also resembled each other very closely.

Serum neutralisation tests in eggs were confined to comparison of 3 strains namely Herts, Massachusetts and California. The results obtained in these tests confirmed the existence of slight differences in antigenic structure between these strains.

V. Electron microscopical appearances.

Comparative studies of electron micrographs of the 8 strains of N.D.V. showed no appreciable differences in their morphology. Study of virus particles at different ionic concentrations of the suspending fluid showed that the particles were susceptible to change in their morphology as a result of osmotic pressure. There was a marked tendency to shrinkage from the spherical to the cylindrical form following exposure of the virus to saline concentrations above 2% suggesting that the virus particles possess a surface osmotic barrier and some form of internal supporting structure.

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